Leaf traits and foliar CO_2 exchange in a Peruvian tropical montane cloud forest

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Declaration

I declare that this thesis has been composed by myself and has not been submitted for any other degree. The work described is my own, except where indicated otherwise.

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Abstract

Tropical montane cloud forests (TMCF) are one of the most fascinating, but least understood ecosystems in the world, and the interest in the carbon (C) cycle of TMCFs with regard to carbon sequestration and storage practices has increased rapidly in recent years. One feature that prevails in all TMCFs is a decrease in aboveground net primary productivity (ANPP) and standing biomass and leaf area index (LAI) with increasing altitude, together with the stunted growth form of the trees. This thesis focuses on the input part of the TMCF C-cycle, and investigates the controlling factors on photosynthesis on a leaf, canopy, and ecosystem level in the Kosñipata valley in south east Peru, on the eastern slope of the Andes (13º11'28''S / 71º35'24''W).

Leaf traits are known to relate to foliar C-exchange, and compared with other altitudinal transect studies of TMCFs, the studied sites had similar altitudinal trends for foliar nitrogen (N) content (though not for phosphorus) and leaf mass per area (LMA), with N content decreasing and LMA increasing with altitude. N concentrations were relatively high and LMA values relatively low, but this observed relationship was consistent with those found in global leaf trait surveys. Examining plant stoichiometry (i.e. N:P ratios), the data suggests that unlike the general hypothesis, the Kosñipata forests are not N limited, except for the study site at 2990 m a.s.l.. At the 2990 m a.s.l. site, which is the focal study site of the thesis, photosynthetic parameters V_{cmax} (the carboxylation efficiency of the Rubisco protein) and J_{max} (the electron transport efficiency) proved to be similar to those found in lowland tropical rainforest leaves when expressed on an area basis and standardised to 25 °C (55.6 ± 2.6 and 106.5 ± 5.2 μmol m^{-2} s^{-1}, for V_{cmax} and J_{max}, respectively). However, when standardised to the mean ambient TMCF temperature of 12.5 °C, both photosynthetic parameters were much lower than ambient tropical rainforest V_{cmax} and J_{max} values. The TMCF J_{max} -V_{cmax} relationships were steeper than found in other tropical biomes, indicating a possible adaptation to the lower light availability in TMCFs because of frequent cloud cover, or a consequence of little atmospheric evaporative demand, which is also due to the humid conditions in this forest type. Although N-V_{cmax} relationships were significant ($P<0.05$), the fit was not very strong

and the relationship between nitrogen use efficiency (NUE) and V_{cmax} indicates that TMCF species can be regarded as a different plant functional type compared with other tropical forest types. Diurnal measurements of net photosynthesis (A), stomatal conductance (g_s) and leaf water potential (Ψ_{leaf}) showed that different TMCF species experienced non-contrasting diurnal patterns of Ψ_{leaf} and g_s in the dry season. The observed patterns suggest that some TMCF species can be classified as isohydric species, while others behave anisohydrically. Additionally, *in situ* g_s was not very responsive to these to the range of experienced photosynthetically active radiation (PAR), vapour pressure deficit (VPD) or soil water content (SWC), leading to the conclusion that in the studied TMCF, drought stress does not play a role in C-uptake. When using the measured photosynthetic parameters for up-scaling C-uptake to stand scale with a Soil-Plant-Atmosphere model, simulated annual gross primary productivity (GPP) was 16.24 ±1.6 T C ha^{-1} yr^{-1}, which is about half the GPP observed in neotropical lowland rainforests. Analyses of the modelled results showed that GPP in this TMCF is mostly controlled by temperature, PAR and leaf area index (LAI) and when increasing these three factors to values found in tropical lowland forest, GPP increased up to 75%. In addition, the modelled results indicate that hydraulic limitations on TMCF C-uptake are very unlikely under current climatic conditions. The modelled results also showed that increases in radiation as a result of less cloud cover do not translate to straightforward increases of GPP. The cloudy conditions of TMCFs, which reduced incident PAR in TMCFs, should therefore not be regarded simply as a negative control on TMCF GPP. Instead, the increase in fraction of diffuse radiation partially offsets the decrease in GPP following the reduction in PAR. Overall, the results of this study show that leaves of Andean TMCF forests have similar C-uptake capacity to tropical lowland rainforests when standardized to similar temperatures, but that for *in situ* C-uptake temperature, radiation and LAI are the key controls.

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List of used abbreviations

A	-	in situ net photosynthesis
ANPP	-	aboveground primary productivity
A_{sat}	-	photosynthesis at saturating light levels
BNPP	-	belowground primary productivity
CV	-	coefficient of variance
GPP	-	gross primary productivity
g_s	-	stomatal conductance
J_{max}	-	electron transport efficiency
$J_{max\text{-}area}$	-	electron transport efficiency on an area basis
$J_{max\text{-}mass}$	-	electron transport efficiency on a mass basis
k	-	extinction coefficient
LAI	-	leaf area index
L_d	-	leaf tissue density
LDMC	-	leaf dry matter content
LMA	-	leaf mass per area
LRF	-	lowland tropical rainforest
MAT	-	mean annual temperature
MCF	-	tropical montane cloud forest
N_a	-	nitrogen per leaf area
N_{area}	-	nitrogen per leaf area
N_m	-	nitrogen per leaf mass
NPP	-	net primary productivity
NUE	-	nitrogen use efficiency (V_{cmax}/N)
PAR	-	photosynthetically active radiation
P_a	-	phosphorus per leaf area
P_{area}	-	phosphorus per leaf area
P_m	-	phosphorus per leaf mass
Q10	-	the factor by which a reaction changes when increasing the temperature 10 °C
R_d	-	dark respiration
$R_{d\text{-}area}$	-	dark respiration on a leaf area basis
$R_{d\text{-}mass}$	-	dark respiration on a leaf mass basis
RH	-	relative humidity

R_l - respiration under light conditions

RMSE - root mean square error

SWC - soil water content

T_{air} - air temperature

T_{leaf} - leaf temperature

TMCF - tropical montane cloud forest

V_{cmax} - carboxylation efficiency of Rubisco

V_{cmax_area} - carboxylation efficiency of Rubisco on an area basis

V_{cmax_mass} - carboxylation efficiency of Rubisco on a mass basis

VPD - vapour pressure deficit

Please note that since Chapter 2 has been published, the abbreviations in this Chapter are different due to the preferences of the publisher.

Chapter 1: General introduction to the thesis

1. 1 Tropical montane cloud forests

Tropical montane cloud forests (TMCF) can be found in tropical mountainous regions all over the world (Figure 1), usually between 1000 to 3000 m a.s.l.. Defining TMCFs is not straightforward, but according to the definition from the Puerto Rico Cloud Forest Symposium, the basic characteristic beyond latitude is the regular presence of clouds and mist associated with mountainous orography (Hamilton *et al.* 1995a). Typical vegetation characteristics when compared with lowland tropical rainforests include: a stunted growth of trees; the sclerophyllous character of the leaves; the high abundance of vascular and non-vascular epiphytes (Figure 2); a lower aboveground productivity; lower leaf area index (LAI); lower litter fall, and particularly high rates of endemism (Hamilton *et al.* 1995b, Foster 2001).

Figure 1. The worldwide distribution of TMCFs (from: Hamilton *et al.* (1995b)).

There is a high interest in the protection and conservation of TMCFs, despite their relatively low abundance in the world, representing on an area basis only 2.5% of the Earth's ecosystems. First, TMCFs have proved to play an important hydrological role in the regions where they occur, feeding lower elevation streams and rivers through substantial capture of horizontal precipitation, also known as fog precipitation or cloud stripping, in addition to the high rainfall they experience (*e.g.*

Bruijnzeel and Proctor 1995, Cavelier *et al.* 1996, Holder 2004). Furthermore, TMCFs are species rich, and contain large numbers of endemic species leading them to be designated as 'global biodiversity hotspots' and hence an ecotype whose protection is considered a very high priority (Gentry 1992, Leo 1995, Myers *et al.* 2000). More than four-fifths (86%) of the TMCFs that are recognized by the UNEP-WCMC are found within the Global 200 Priority Forest Eco-regions identified by the World Wildlife Fund (Bub *et al.* 2004). In addition, the interest in the C-cycle of TMCFs with regard to carbon sequestration and storage practices has increased rapidly in recent years (*e.g.* Fehse *et al.* 2002, Martinez *et al.* 2009).

Figure 2. Some examples of typical TMCF vegetation in the Kosñipata valley in Peru.

The largest threats to TMCFs are land use change and predicted climate change (Loope and Giambelluca 1998, Foster 2001, Enquist 2002, Nair *et al.* 2003, Hemp 2009). Land use change involves both pressure on the TMCF ecosystem from lower altitudes where forests are often cleared for conversion into coffee or sugar cane plantations, or for cattle ranching; whilst at higher elevations pressure on TMCFs is often exerted because of the need for grassland and its management through burning by pastoralists (Sarmiento and Frolich 2002, Martinez *et al.* 2009). As for climate change, it is expected that the optimum climatic conditions for TMCFs will increase in altitude several hundreds of meters the coming century (Still *et al.* 1999) or will disappear all together (Williams *et al.* 2007), resulting in a replacement of TMCFs by other types of ecosystems. Studies on frogs and toads in Costa Rica have already showed local extinction of species, through changes in mist frequencies following

sea surface temperature changes (Pounds *et al.* 1999), while the migration rate of TMCF tree species has been shown to be less rapid than the up slope trend in climate in the Peruvian Andes (Feeley and Silman, in review). Furthermore, recent studies have already shown an increase of altitude of the freezing level in the tropical Andes (Bradley *et al.* 2009) and temperatures in this region are expected to increase, whilst precipitation might decrease (Vuille *et al.* 2003, Urrutia and Vuille 2009).

1.2 Productivity in TMCFs

Whilst the conservation value of TMCFs has been widely recognized, detailed understanding of the functioning of these forest ecosystems remains an ecological frontier. Indeed, the explanation of the apparent low productivity of TMCFs has challenged ecological thinking for a long time (*e.g.* Grubb 1977, Vitousek 1984, Tanner *et al.* 1998, Vitousek 1998), most probably because of a lack of detailed process-level data and understanding. Specifically, the cited low productivity of these forests has tended to have referred to the aboveground component of productivity in TMCFs. This observation has often been made alongside a recognition of lower standing biomass with increasing altitude, together with the stunted growth form of the trees at higher elevations (Bruijnzeel and Veneklaas 1998, Waide *et al.* 1998, Kitayama and Aiba 2002, Moser *et al.* 2007). Many explanations have been put forward to explain these features, and most of them can be attributed to the different environmental conditions that TMCFs experience compared to tropical rainforest at lower altitudes. First, like other mountainous ecosystems in the world, TMCFs experience lower temperatures compared with other forests found at similar tropical latitudes (*e.g.* Caldwell *et al.* 1980, Barry 1981). Secondly, the frequent immersion in clouds causes a decrease in the fraction of direct photosynthetically active radiation (PAR), but an increase in the fraction of diffuse PAR (Letts and Mulligan 2005, Moser *et al.* 2007), and an increase in the relative humidity (Grubb and Whitmore 1966, Bruijnzeel and Proctor 1995). Finally, with increasing altitude, nutrients are presumed to be less available because of lower mineralization rates as a consequence of lower temperatures and lower densities of invertebrates (Olson 1994, Richardson *et al.* 2005, Illig *et al.* 2008). In this view,

nitrogen (N) is especially less available to TMCF plants compared with phosphorus (P) because microbial N-fixation is inhibited, while P is less affected, because tropical soils of intermediate age are known to have higher P availability than old, undisturbed tropical soils (Tanner *et al.* 1998, Pearson and Vitousek 2002, Porder *et al.* 2007).

The three factors described above (*i.e.* low temperature, low PAR intensities and low N availability) have been hypothesized to be the main factors limiting TMCF productivity (Grubb 1977, Bruijnzeel and Veneklaas 1998, Tanner *et al.* 1998, Vitousek 1998, Letts and Mulligan 2005, Raich *et al.* 2006). Other hypotheses include periodic water shortages because of the often shallow soils, water logging of the soils because of low atmospheric evaporative demand, and high phenolic concentrations of the TMCF litter that reduce decomposition and hence nutrient availability, although these latter hypotheses have been considered to be of less importance (Bruijnzeel and Veneklaas 1998).

When it comes to measuring the productivity in TMCFs, most research has been performed on aboveground parts of the TMCF C-cycle. Measurements of stem growth, litter fall, and leaf are index (LAI) have shown lower aboveground standing biomass and lower aboveground net primary productivity (ANPP) in TMCFs compared with lowland rainforests (*e.g.* Grubb and Whitmore 1966, Proctor *et al.* 1989, Veneklaas 1991, Bruijnzeel and Veneklaas 1998, Kitayama and Aiba 2002, Moser *et al.* 2007). In recent years, however, the belowground net primary productivity (BNPP) of TMCFs has received more attention and studies have demonstrated either increases in fine root biomass and root productivity with altitude (Roderstein *et al.* 2005, Leuschner *et al.* 2007), or a lower BNPP in TMCFs compared with lowland rainforest without an apparent decrease or increase in the cloud forest belt (Girardin *et al.*, in press). In addition to these BNPP observations, the increase in soil organic C with elevation in TMCF ecosystems together with constant soil respiration rates (Zimmermann *et al.* 2009) suggest a substantial below-ground allocation of productivity. Clearly the TMCF C-cycle requires further investigation, and yet very little is known on the basic process of production, the

actual rates of photosynthesis at leaf and especially canopy scale, and hence of gross primary productivity (GPP) in TMCFs.

1.3 Photosynthesis and gross C-uptake in TMCFs

The C-uptake of a canopy depends on a variety of factors, ranging from the inherent photosynthetic capacity of the leaves, the canopy structure (*e.g.* LAI and leaf angles) and environmental controls (*e.g.* temperature, VPD, radiation flux density and quality). In this paragraph, I briefly discuss these factors in the context of TMCFs.

1.3.1 Leaf level photosynthesis

Based on global leaf trait relationships (*e.g.* Wright *et al.* 2004, Reich *et al.* 2007), the leaf level photosynthetic capacity would be expected to be relatively low in TMCFs, since TMCF foliar nutrients are lower and leaf mass per area (LMA) higher than tropical forests from lower altitudes (*e.g.* Tanner *et al.* 1998, Kitayama and Aiba 2002, van de Weg *et al.* 2009). This is because within the 'global leaf economic spectrum' (sensu Wright *et al.* 2004), leaves with low nutrient content invest their scarce resources in structural features like a high LMA to increase their longevity, and less in their photosynthetic apparatus (Shipley *et al.* 2006). However, on a leaf level, photosynthetic rates in TMCFs at saturating light (A_{sat}) have been shown to vary from 3.1 to 10.2 μmol m^{-2} s^{-1} in TMCFs at altitudes ranging from 600 and 3700 m a.s.l. in Ecuador, Colombia and Malaysian Borneo (Hikosaka *et al.* 2002, Letts and Mulligan 2005), which is within the range observed in lowland tropical rainforest (Carswell *et al.* 2000, Domingues *et al.* 2005, Domingues *et al.* 2007, Meir *et al.* 2007). This leaves it unclear how well the TMCF foliage compares with the known global relationship on leaf traits and C-uptake.

Furthermore, next to the scarce research on TMCF A_{sat}, no data are available on the biochemical determinants of A_{sat}, the maximum carboxylation velocity of the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (V_{cmax}) and the maximum rate of electron transport (J_{max}) in TMCFs. Both V_{cmax} and J_{max} give a better indication of the photosynthetic capacity of a leaf than A_{sat}, because they are

independent of the ambient stomatal conductance (g_s) or mesophyll conductance (g_m), which makes them much more useful as modelling parameters (Kattge *et al.* 2009) and often more suitable to aid comparisons among different ecosystems or species than A_{sat}. Additionally, V_{cmax} and J_{max} are used in the well tested Farquhar equation for leaf-level C_3 photosynthesis (Farquhar *et al.* 1980, von Caemmerer and Quick 2000). This equation is used in many global terrestrial biosphere models (GTM) as well as in models that operate on a finer scale (*e.g.* Williams *et al.* 1998, Knorr and Heimann 2001, Zaehle *et al.* 2005, Alton and North 2007, Kattge *et al.* 2009, Mercado *et al.* 2009). As the relationship between leaf nutrient content and V_{cmax} or J_{max} is uncertain, especially for the tropics (Kattge *et al.* 2009, Domingues *et al.* 2010), field-based estimates of V_{cmax} and J_{max} are essential in order to make good predictions of C-uptake by TMCFs on a canopy or landscape scale.

1.3.2 Canopy structure and C-uptake

When scaling from leaf to canopy, canopy structure and the investment in photosynthetic capacity throughout the canopy have been shown to be important in determining the C-uptake in a stand (*e.g.* Niinemets 2007, Toda *et al.* 2009). In general, canopy profiles from both temperate and tropical forests show higher A_{sat} in the top part, where leaves receive more radiation, than in lower, less light-receiving parts (*e.g.* Niinemets 1997, Kull 2002, Meir *et al.* 2002, Domingues *et al.* 2005). However, when analysing the patterns of A_{sat}, foliar N and P or *LMA* throughout a canopy, their relationships with height are not as tight as the optimisation theory of Field (1983) predicts (DeJong and Doyle 1985, Meir *et al.* 2002, Anten 2005). Reasons for the "non optimal" distribution have been attributed to different causes, ranging from strategies to avoid herbivory or to cope with changing light environments, to inevitable consequences of a leaf's life history and the costs associated with relocating nutrients (Stockhoff 1994, Warren *et al.* 2000, Niinemets *et al.* 2007). However, probably most important in explaining the "non optimal" distribution of photosynthetic capacity throughout the forest canopy are the limits of what combinations of *LMA* and N content species can realistically achieve (Meir *et al.* 2002). Lloyd *et al.* (2009) showed that for tropical canopies, the distribution of

A_{sat} throughout the canopy (or V_{cmax} and J_{max} that determine this parameter) is probably dependent on a combination of A_{sat} and LAI, with canopies that have a high A_{sat} showing steeper gradients than those with a lower A_{sat} at the top of the canopy. Although datasets on TMCF foliar nutrients and *LMA* are available, little is known about their distribution throughout the TMCF canopy, since most studies have only included fully sunlit leaves, leaving a gap in our understanding of TMCF canopy profiles.

1.3.3 Environmental controls on photosynthesis

Climatic factors like temperature, light quality and quantity and air humidity only occasionally occur at optimum conditions for photosynthesis. These factors can all down-regulate CO_2 assimilation such that photosynthesis rarely occurs at A_{sat} (Paul and Foyer 2001). In TMCFs it is most likely that temperature and light play an important role in controlling C-uptake, rather than hydraulic constraints (Bruijnzeel and Proctor 1995; Bruijnzeel and Veneklaas 1998). Limited studies on TMCF sap flow and transpiration have shown no evidence of 'midday depression' (the closure of stomata, often soon after midday, when high vapour pressure deficits are experienced) and significant positive relationships between incident radiation and TMCF sap flow have been observed (Zotz *et al.* 1998, Motzer *et al.* 2005). This suggests that on days with relatively little cloud cover, atmospheric demand is rarely so high as to limit photosynthesis through stomatal closure.

As for light conditions, Bruijnzeel *et al.* (1993) showed 21% lower PAR levels in a TMCF compared to montane forests growing below the level of the cloud belt in Malaysia. Lets and Mulligan (2005) also showed a decrease of up to 50% of PAR received by the canopy in TMCFs than in lower-elevation rainforests in Columbia. However, in addition to attenuation of the amount of PAR, mist and clouds also scatter radiation, leading to diffuse light conditions in TMCFs. Under diffuse light, radiation can enter the canopy from all directions, which has been shown in a range of vegetation types to increase the gross C-uptake of the whole canopy, especially understorey species (Roderick *et al.* 2001, Johnson and Smith 2006, Alton *et al.*

2007). However, for tropical lowland rainforests, decreases in gross photosynthesis under cloudy conditions, or under higher diffuse light conditions after biomass burning have been reported (Graham *et al.* 2003, Yamasoe *et al.* 2006). In TMCFs, the importance of diffuse light for GPP has not been investigated so far.

Temperature effects on foliar CO_2 exchange are very dependent on the time scale at which temperature changes take place. Respiration and photosynthesis are both enzymatic processes, so intuitively one would expect them to increase with increasing temperatures and in general, especially near the ambient-acclimated optimum, respiration is more sensitive to temperature changes than photosynthesis (Berry and Bjorkman 1980; Atkin *et al.* 2005; Campbell *et al.* 2007). It is important, however, to distinguish between the short-term and long-term sensitivities of respiration and photosynthesis to temperature. As for short-term responses, the temperature sensitivity of photosynthesis and respiration can differ among growth temperature, seasons and species (Berry and Bjorkman 1980, Tjoelker *et al.* 2001, Stitt and Hurry 2002, Atkin *et al.* 2005, Atkin *et al.* 2006, Sage and Kubien 2007, Zaragoza-Castells *et al.* 2007). However, after a long-term change of growth temperature, a variety of plant species have shown to acclimate their photosynthesis levels to an extent where no significant differences in photosynthesis between the initial and final growth temperatures can be observed (Atkin *et al.* 2006; Campbell *et al.* 2007). For respiration, this acclimation is less complete, but still occurs (Atkin *et al.* 2006, Campbell *et al.* 2007, Zaragoza-Castells *et al.* 2007). At a larger scale, global analyses of different ecosystems have indicated little influence of mean annual temperatures (MAT) on NPP, when the growth rates were corrected for the duration of the growing season (Kerkhoff *et al.* 2005). The long-term and short-term importance of temperature effects in TMCFs are still unclear, hence there is a need for a better understanding of the temperature responses by photosynthesis and respiration in these ecosystems.

1.3.4 Modelling TMCF GPP

By combining structural vegetation parameters (*e.g.* photosynthetic capacity, LAI) and soil parameters (*e.g.* root biomass, soil porosity) with meteorological data and well known eco-physiological models on C-uptake and tree hydraulic functioning, it is possible to predict the GPP of an ecosystem at canopy or landscape levels. Although GPP can only be estimated (and not really measured) using models, for TMCFs, this mode of estimating GPP becomes especially relevant, because *in situ* observations of stand-scale net CO_2 fluxes are limited by practical limitations in TMCFs. In mountainous terrain, like TMCFs, using the eddy covariance technique becomes very difficult (Kaimal and Finnigan 1994). Only one modelled estimate of TMCF GPP has been reported. Wang *et al.* (2003) predicted annual GPP values of 60.32 – 24.08 t C ha^{-1} yr^{-1} in tropical forests over an altitudinal range of 450-1050 m a.s.l. respectively, using a canopy process model driven with simulated climate data from a topographical climate and remotely sensed LAI data derived from NDVI measurements. However, no field data on V_{cmax} and J_{max} , or field data on the TMCF canopy structure was included in this study, and their model overestimated GPP up to 43% compared with field observations (24.08 vs. 16.78 t C ha^{-1} yr^{-1}). In sum, estimates of TMCF gross C-uptake - the largest single flux in the terrestrial carbon cycle -, hardly exist and represent a substantial gap in our understanding of TMCF ecology.

1.4 Motivations for this study

The research for this PhD project was part of the Andes Biodiversity and Ecology Research Group (http://www.andesconservation.org). This is a consortium of scientists from the US, UK and Peru studying the eco-physiology, biogeochemistry and the structural and diversity-related properties of the forest vegetation in the Kosñipata valley in Peru (13°S, 70°W). As the preceding sections show, there are components of TMCF C-cycle that are poorly understood, or hardly studied, even though the interest in the forests of this region is growing. Furthermore, although previous studies on TMCFs have focused on productivity or C-cycling in TMCFs in for example Hawaii, Costa Rica, Puerto Rico and Borneo, Andean TMCFs have

received relatively little attention until recently with some reports from Ecuadorean rain forests (*e.g.* Roderstein *et al.* 2005, Leuschner *et al.* 2007, Moser *et al.* 2007, Soethe *et al.* 2008), though very little from other countries in the region. This PhD has addressed a number of questions concerning TMCF photosynthesis, foliar respiration, leaf trait relationships, and environmental controls on TMCF C-uptake. It is a stand-alone study, but also contributes to the overall goals of the ABERG consortium.

1.5 Research area

A large part of the fieldwork that forms the basis for this PhD was performed in previously established 1 ha plots in the Kosñipata valley in south-eastern Peru (13°S, 71°W). In the Kosñipata valley, the base of the cloud zone is located at 1500 - 1800 m a.s.l.. Above this altitude, the cloud forest zone is continuous to approximately 3400 m a.s.l., while above this altitude "puna" grasslands are the main vegetation type. Two additional 1-ha lowland tropical rainforest plots in the Madre de Dios region that were established by Phillips *et al.* (1998) were included in the study discussed in Chapter 2. Together, the plots form an altitudinal gradient that traverses from lowland tropical rainforest, lower and upper montane cloud forest, to the "puna" grasslands above the tree line.

The main focal site of this study (Chapters 3-5 of this thesis) is a TMCF site at 2990 m a.s.l., that is located near the Wayquecha research station (13º11'28''S / 71º35'24''W), owned by the non-governmental organization Amazon Conservation Association **(**in Spanish: Asociación para la Conservación de la Cuenca Amazónica**).** A summary of the mean annual temperatures (°C), mean annual soil temperatures (°C), and annual precipitation (mm $year^{-1}$) throughout the Kosñipata valley can be found in Table 1.

Table 1. Mean annual air temperature (ºC), soil temperature at 10 cm depth (ºC), and annual precipitation rates (mm $year^{-1}$) along the studied altitudinal transect discussed in this thesis. Means are based on continuous measurements at the sites from September 2006 – September 2008.

Elevation (m)	Mean annual air temperature (°C)	Mean annual soil temperature (°C)	Annual precipitation (mm yr^{-1})
220	26.4	24.5	2730
1000	20.7	20.7	3087
1500	18.8	19.06	2631
1855	18.03	17.4	2472
2990	12.5	12.5	1706

1.6 Structure and set up of the thesis

This thesis is structured as a series of papers that have been published (Chapter 2), or are intended for publication (Chapter 3-5), with a general discussion and conclusion section included as a final chapter (Chapter 6), in which some suggestions for further research are discussed as well. As a consequence of this structure, abbreviations used in the chapters may vary, depending on the preference of the journals, but all abbreviations can be found in the list of abbreviations given at the start of the thesis. The overall aim of this PhD was to understand what controls the C-uptake in a Peruvian TMCF. In order to do so, the four separate main chapters address this question on a leaf, canopy and ecosystem level (Figure 3), with the order of the chapters following this hierarchy. Chapter 2-4 discuss data collected in the field, while in Chapter 5 is the product of a modelling exercise using part of this data. Below, the main aims and outline of each paper (Chapter 2-5) are discussed.

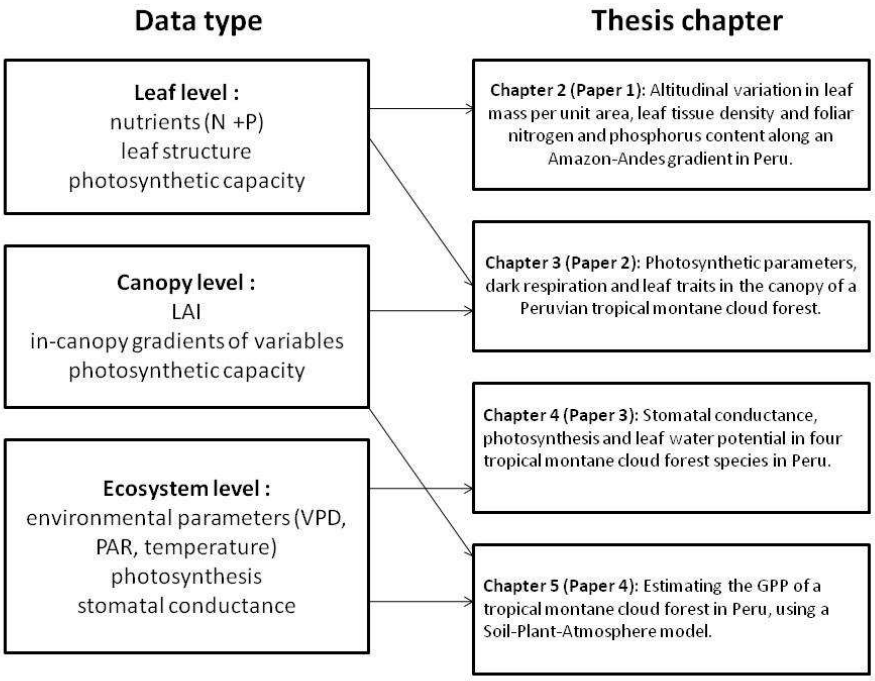

Figure 3. Schematic overview of data types that were collected for this thesis and for which chapters they were used.

1.6.1 Chapter 2 (Paper 1): Altitudinal variation in leaf mass per unit area, leaf tissue density and foliar nitrogen and phosphorus content along an Amazon-Andes gradient in Peru

The main aims of this paper were:

- To investigate how foliar nutrient content (N and P) on a mass and area basis and LMA change with altitude in a transect from lowland rainforest to above the TMCF tree line.
- To test whether the hypothesis suggested by Tanner *et al.* (1998), describing that in TMCFs N-limitation plays are more important role than P-limitation, holds for East Andean TMCFs.
- To test whether the observed TMCF relationships between leaf traits (*LMA* are consistent with those observed in global surveys (Wright *et al.* 2004, Shipley *et al.* 2006).

Global leaf traits relationships are important in determining the C-uptake capacity of a plant. In this study, the changes in (LMA), nitrogen (N) and phosphorus (P) content of leaves from the most abundant species along an altitudinal gradient (3600-220 m) along the Kosñipata valley in Peru. The results were compared to other tropical altitudinal gradients reported from Borneo, Hawaii, Puerto Rico that range from tropical lowland rainforests to tropical montane cloud forests (TMCF). LMA increased with altitude, though overall LMA values were lower than comparable altitudinal tropical gradients. N content showed a decreasing, if patchy, trend with altitude. N contents were higher than in comparable studies, though these high N values, combined with low LMA values fit in well with the global leaf economic spectrum. P content results did not show an altitudinal trend, but instead demonstrated the spatial variability amongst tropical leaves. Finally, although P limitation in the leaves did decrease with altitude, in none of the investigated field sites the leaves suggested N limitation, except for the field site at 2990 m a.s.l..

1.6.2 Chapter 3 (Paper 2): Photosynthetic parameters, dark respiration and leaf traits in the canopy of a Peruvian tropical montane cloud forest.

The main aims of this paper were:

- To provide a first quantification of V_{cmax} and J_{max} in TMCFs, and to compare them with values from other tropical forests, both in terms of absolute values, and the relationship between them.
- To describe how the leaf parameters (V_{cmax}, J_{max}, dark respiration (R_d), foliar N and P and LMA) are distributed throughout the TMCF canopy and to analyze what part of their variance is explained by sampling height within the canopy, or intra- and inter-species differences.
- To investigate how the foliar CO_2 exchange parameters V_{cmax}, J_{max} and R_d scale with foliar N and P and LMA, both on a mass and area basis.

There are few studies available that describe TMCF photosynthesis, and virtually no data can be found on the photosynthetic parameters V_{cmax} and J_{max} in TMCFs, or their relationship to other leaf traits. Therefore, the V_{cmax} and J_{max}, foliar dark respiration (R_d), foliar nitrogen (N) and phosphorus (P) and leaf mass per area (*LMA*) at different canopy levels in a TMCF at 2990 m a.s.l. were quantified. The photosynthetic parameters proved to be similar to those found in lowland tropical rainforest leaves when expressed on an area basis and corrected to 25 °C (55.6 ± SE 2.6 and 106.5 ± SE 5.2 μmol m^{-2} s^{-1}, for V_{cmax} and J_{max}, respectively), while dark respiration was relatively high (1.43 ± 0.06 μmol m^{-2} s^{-1}) when standardized to 25 °C. Scaled to the *in situ* mean annual temperature (12.5 °C), however, V_{cmax} and J_{max} were considerably lower (17.45 ± SE 0.82 and 50.51 ± SE 2.5μmol m^{-2} s^{-1}, respectively), as well as R_d (0.57 ± SE 0.02 μmol m^{-2} s^{-1}). The relationships of the leaf traits with canopy height were similar to those observed in other forest canopies, though for V_{cmax} and J_{max} a larger part of the variance was explained by inter-species differences than for R_d. Finally, the photosynthetic parameter V_{cmax} did not have any significant relationships with other leaf traits, except for N, and this fit was relatively noisy. This latter observation, however, supports the hypothesis that this TMCF is N limited, while the V_{cmax}:*N* ratio suggests TMCFs qualify as a distinct plant functional type among tropical ecosystems.

1.6.3 Chapter 4 (Paper 3): Stomatal conductance, photosynthesis and leaf water potential in four tropical montane cloud forest species in Peru.

The main aims of this paper were:

- To investigate the diurnal patterns of stomatal conductance (g_s), leaf water potential (Ψ_{leaf}) and net photosynthesis (*A*) for four abundant TMCF species.
- To test how TMCF g_s relates to the experienced ranges in leaf water status and changes in environmental variables like vapour pressure deficit (VPD), leaf temperature (T_{leaf}), soil water content (SWC) and photosynthetic active

radiation (PAR), and to additionally test whether this has consequences for TMCF C-uptake.

For four dominant TMCF species at the focal site at 2990 m a.s.l., measurements of g_s, Ψ_{leaf} and A were collected throughout the daytime during the dry season of 2008. In two of the four investigated species, Ψ_{leaf} stayed close to 0 throughout the day, although g_s for the same leaves showed a diurnal pattern, resembling stomatal behaviour of so called 'isohydric' species. The other two species had diurnal responses of Ψ_{leaf} and g_s similar to 'anisohydric species', showing that TMCF species can have different stomatal strategies when growing under the same environmental conditions. None of the investigated species experienced a midday depression of g_s or A. The lack of statistical difference in g_s between 'sun' and 'shaded' leaves, contrary to the statistical difference in A between 'sun' and 'shade' leaves indicate that C-uptake in these leaves was not primarily controlled by g_s. Furthermore, g_s was hardly responsive to ambient changes in VPD or SWC for all of the species. It could be that the ranges of soil water availability and VPD experienced in this study do not cover a range large enough to detect relationships between environmental parameters and g_s. Nevertheless, it implies that even in the dry season, the studied TMCF is not limited in C-uptake through drought stress.

1.6.4 Chapter 5 (Paper 4): Estimating the GPP of a tropical montane cloud forest in Peru

The main aims of this paper were:

- To simulate TMCF gross primary productivity (GPP) and compare it with known values from lowland tropical rainforests.
- To determine what the key controls (structural or environmental) on GPP are in a TMCF, and to what extent they can explain observed differences between TMCF and tropical lowland GPP.
- To investigate how the ratio of diffuse and direct radiation affects TMCF GPP.

For this study, the GPP of a TMCF at 2990 m a.s.l. was simulated by using the Soil-Plant-Atmosphere (SPA) model developed by Williams (1996). Physiological and

structural parameters of the vegetation and soil that were collected in the field were combined with a one year meteorological data set. Simulated annual GPP was 16.24 ± 1.6 t C ha^{-1} yr^{-1}, about half the GPP observed in neotropical lowland rainforests. Analyses of the modelled results showed that natural variation in GPP in this TMCF is mostly controlled by temperature, PAR and LAI. This latter factor is only of importance if the photosynthetic capacity per leaf area stays constant. In other words, if the TMCF would increase its LAI, it needs to increase the amount of nutrients in its canopy concurringly to increase the GPP, which provides indirect support for the hypothesis that TMCFs are N limited. When temperature, PAR and LAI were increased to values as found in lowland tropical forests, GPP increased up to 75%. As for radiation, higher radiation levels increase GPP if the diffuse portion of irradiance stays the same. However, since increases in radiation are the result of less cloudiness, this also results in less diffuse radiation, and lower proportions of diffuse light decreased GPP at any given average irradiance level. Therefore, it was concluded that the cloudy condition of TMCFs should not be regarded simply as a negative control on TMCF GPP by reducing the total irradiance, since the increase in diffuse radiation partially offsets the loss in GPP. Furthermore, the simulated TMCF was very insensitive to changes in the hydraulic parameters, or changes in soil water content, which provided more evidence that under current conditions, (periodic) drought stress is a very unlikely explanation for the lower GPP for this TMCF.

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Chapter 2

Paper 1: Altitudinal variation in leaf mass per unit area, leaf tissue density and foliar nitrogen and phosphorus content along an Amazon-Andes gradient in Peru

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Supplementary results accompany this chapter (Appendix I)

Abstract

Background: Leaf traits are important in determining the capacity of a plant to acquire carbon, but few data are available for montane cloud forests in the Andes.
Aims: To investigate the changes in leaf traits along a large altitudinal transect (220-3600 m) from lowland to montane cloud forest in Peru.
Methods: We measured leaf mass per area (*LMA*, g m^{-2}), leaf tissue density (L_d, g cm^{-3}) and foliar nitrogen (N) and phosphorus (P) content, both on a mass (N_m and P_m, %) and area (N_a and P_a, g m^{-2}) basis for the most abundant species locally.
Results: *LMA* increased with altitude (62.8-169.4 g m^{-2}), though overall, *LMA* was lower than in comparable tropical elevation gradients. N_m declined significantly with altitude (2.39-1.25 %, $P < 0.05$), but N_m contents were higher than in comparable studies. The relatively high N_m and low *LMA* values are consistent with published global leaf trait datasets. No altitudinal trend for P_m was found; rather, our data highlighted the spatial variability in P_m (and P_a) within and among sites at different elevations. Foliar N:P ratios did not show a trend with altitude and did not indicate N limitation except at 3000 m altitude.
Conclusions: Though leaf traits showed altitudinal trends similar to other studies, contrary to the general hypothesis, our data suggest that the tropical montane forests presented here are not N limited.

Keywords: Elevation gradient, foliar nutrients, leaf mass per area, leaf traits, nitrogen, phosphorus, tropical montane cloud forests

2.1 Introduction

Along an altitudinal gradient from a tropical lowland rain forest (LRF) to a tropical montane cloud forest (MCF), climate, soils, and vegetation change remarkably. Intrinsic to all mountainous areas are decreases in air temperature, atmospheric pressure and air density relative to lower altitudes (Barry 1981), together with increases in solar radiation fluxes and a higher UV fraction as a result of decreased atmospheric turbidity (Caldwell 1968; Barry 1981). In the tropics, mountainous areas also follow these patterns, although they experience high diurnal variations in some variables (*e.g.* temperature), and little seasonal variation (Rundel 1994). MCFs differ from other tropical mountain ecosystems because they are also frequently immersed in clouds, the feature after which they are named (Stadtmüller 1987). The elevations at which these cloud events occur, and as a consequence MCFs, differ amongst tropical regions around the world. Various climatic and geographical factors that influence both cloud formation (*e.g.* presence and water currents of the nearest sea, trade winds inversions) and vegetation patterns (*e.g.* the Massenerhebung effect (Grubb 1971)), macro and micro-relief patterns of the mountain ranges) determine at what altitude MCFs prevail (Stadtmüller 1987; Hamilton *et al.* 1995). Among the consequences of immersion in clouds is a decrease in direct photosynthetic active radiation (PAR), but an increase in diffuse PAR (Letts and Mulligan 2005; Moser *et al.* 2007), and an increase in the relative humidity (Grubb and Whitmore 1966; Bruijnzeel and Proctor 1995; Bruijnzeel and Veneklaas 1998; Foster 2001).

The visually most striking differences between LRF and MCF vegetation include a shift from straight tall trees to trees with a much shorter and stunted stature, an increase in the presence of vascular and non-vascular epiphytes, together with a decrease in above-ground biomass, as well as a decrease in leaf area index (LAI) (Grubb and Whitmore 1966; Veneklaas 1991; Bruijnzeel and Veneklaas 1998; Roderstein *et al.* 2005; Leuschner *et al.* 2007; Moser, *et al.* 2007). Less obvious forest characteristics, such as leaf traits, also differ between LRFs and MCFs. Generally, the foliar nutrient concentrations per dry weight biomass of phosphorus (P) and nitrogen (N) decline with altitude, while the leaf mass to area ratio (*LMA*)

increases (Grubb 1977; Vitousek *et al.* 1992; Tanner *et al.* 1998; Kitayama and Aiba 2002; Soethe *et al.* 2008). However, the leaf traits in MCFs in the Andes have been little studied, leaving a gap in the present understanding of MCF functioning.

Observed relationships between physical and chemical leaf traits and basic physiological processes such as photosynthesis and respiration across the world's plant species show the macronutrients N and P to be of major importance in influencing foliar carbon (C) uptake capacity (Reich *et al.* 1992; Wright *et al.* 2004; Reich *et al.* 2009). Photosynthesis is an N-intensive process, because of its high N requirement by the carboxylation enzyme Rubisco and related light harvesting complexes. According to Chapin (1991), a 'sun leaf' of a C3 plant invests up to 26% of its N in CO_2 fixation (Rubisco) and 19% in the light harvesting complexes. Nitrogen content is therefore generally correlated with photosynthetic capacity (Field and Mooney 1986; Evans 1989). Phosphorus is important for photosynthesis in recycling ribulose-1,5-biphosphate, (RuBP), in ATP transformation and in the synthesis of new proteins and nucleic acids (RNA and DNA) (Sterner and Elser 2002). Hence, N and P are of particular importance for plant growth, though their relative importance appears to be different amongst biomes (Ågren 2004; Hessen *et al.* 2004) and the relationship between N and photosynthesis varies with different levels of available P (Reich *et al.* 2009). Along with leaf nutrients, *LMA* is also strongly correlated with photosynthesis and respiration (*e.g.* Meir *et al.* 2001). High *LMA* values generally occur in leaves with a high longevity, low nutrient contents and low instantaneous CO_2 uptake capacities (Reich *et al.* 1998; Wright, *et al.* 2004; Poorter *et al.* 2009). The development of a high *LMA* is sometimes considered a strategy to increase the longevity of a leaf, in order to optimise the use of scarce nutrients (*e.g.* Kikuzawa 1991; Reich *et al.* 1992; Reich 1993).

On a global scale, tropical foliage generally has low P concentrations, compared to their N status (Reich and Oleksyn 2004; Kerkhoff *et al.* 2005; Townsend *et al.* 2007). McGroddy *et al.* (2004) found a higher mean ± SE N:P ratio in tropical leaves (43.3 ± 4.6, n = 7) compared with temperate broad leaf foliage (28.2 ± 1.5, n = 28), although the variability in foliar N:P between different tropical sites and seasons

differ up to 25% (Townsend *et al.* 2007) and the slope of the bivariate relationship between N and P differs significantly between sites with low and high fertility in Amazonia (Fyllas *et al.* 2009). The low P content in tropical leaves could be explained by low P availability in tropical soils, which tend to be old and highly weathered (Sanchez 1979; Wardle *et al.* 2004). In addition, Kerkhoff *et al.* (2005) suggested that the relatively high foliar P concentrations in temperate zones compared with tropical areas may be a consequence of higher investment of P in ribosomes to increase biosynthesis to compensate for reduced photosynthesis due to lower temperatures. Furthermore, inorganic P plays a pivotal role in maintaining photosynthesis at low temperatures since low temperatures reduce phosphate recycling (Savitch *et al.* 1997; Strand *et al.* 1997). Because in lowland tropical forests photosynthesis and phosphate recycling are less limited by temperature, a smaller investment in ribosomal P and inorganic P might be sufficient, leading to smaller foliar P content. Previous studies have shown that when increasing in altitude from LRF to MCF, foliar N and P concentrations both tend to decline, while *LMA* increases (Vitousek *et al.* 1992; Tanner *et al.* 1998; Kitayama and Aiba 2002; Soethe *et al.* 2008). The decrease with altitude in foliar nutrient concentrations is mostly attributed to lower nutrient availability resulting from low mineralization rates at higher altitudes, a decreased nutrient uptake rate by roots because of low temperatures, and low abundance of mycorrhizal fungi which reduces the nutrient supply through this route (Leuschner *et al.* 2007). Furthermore, high humidity in cloud forests might cause low transpiration rates, which would limit the transport of nutrients from the soil to the leaves (Bruijnzeel and Proctor 1995, Bruijnzeel and Veneklaas 1998), although this hypothesis is questioned by studies demonstrating no limitation in transpiration rates in MCFs (Zotz *et al.* 1998; Motzer *et al.* 2005).

Overall, observations show that foliar N and P in MCFs are lower than in LRFs, though the mechanisms explaining these differences are not fully understood. Given the strong links between metabolic activity and leaf chemistry/structure, it is essential that we develop a full understanding on the extent to which the altitudinal gradients in the Andes impact on leaf N:P stoichiometry and structure of leaves in LRFs and MCFs. A fertilization experiment in a Venezuelan MCF showed increased

productivity after N addition (Tanner *et al.* 1992) and Tanner *et al.* (1998) proposed a general rule that LRFs are P limited in their growth while MCFs are limited by N. In this view, MCFs can be compared with temperate forests, where low temperatures cause N mineralization rates to be low and hence limit growth, while the low P availability in LRFs due to the generally weathered and leached status of the soil causes the LRFs to be P limited. However, because of the few data on physical and chemical leaf traits in MCFs, it is unclear to what extent the hypothesis of Tanner *et al.* (1998) is generally applicable. Furthermore, although it has been shown that foliar N and P, and *LMA* tend to correlate strongly on a global scale (Wright, *et al.* 2004; Shipley *et al.* 2006; Reich *et al.* 2007), MCF species were absent from these studies, leaving a substantial gap in knowledge. Since environmental factors such as temperature and the UV:PAR ratio and direct/diffuse irradiance change dramatically over tropical elevation gradients, it is possible that leaf trait and nutrient relationships also differ from those observed in LRFs.

Since few data are available for leaf traits in Andean MCFs and previous studies rarely included data from below or above the typical cloud forest ecotone, we investigated the patterns in foliar N and P content and *LMA* of the locally most abundant species throughout an altitudinal transect in south-east Peru, which transverses from LRF (220 m a.s.l.) through the MCF to the shrubby grasslands with some dispersed trees at 3600 m a.s.l., just above today's tree limit in this area. We tested the following hypotheses: (1) Foliar nutrient content (N_m and P_m) declines with increasing altitude, while *LMA* increases. Consequently, N_a and P_a decrease less with altitude, because of increases in *LMA*. (2) Foliar N_m decreases more with altitude than P_m, leading to N-limitation at high altitudes and P-limitation at low altitudes, as suggested by Tanner *et al.* (1998). (3) Relationships between leaf traits (*LMA* and N_m) in MCF forests are consistent with those observed in global surveys (Wright *et al.* 2004, Shipley *et al.* 2006). Finally, we discuss the absolute values of foliar N, P and *LMA* from other altitudinal MCF transects to see how Peruvian MCFs compare with previous studies.

2.2 Methods

2.2.1 Research Sites

Leaf material was collected from eight different sites in Eastern Peru along an elevation transect (220-3600 m a.s.l.) from the Madre de Dios region in Peru (220 m a.s.l.) to the head of the Kosñipata Valley (Table 1). The two 1-ha plots in the Madre de Dios region were established by Phillips *et al.* (1998). Four 1-ha plots in the Kosñipata valley were established for inventory purposes and are described by M.R. Silman *et al.* (unpublished manuscript), one plot was established in 2007 (S. Saatchi *et al.* unpublished data) and the site at 3600 m a.s.l. was not pre-established. The research sites in the Kosñipata Valley were all montane forests except for the highest elevation plot, at 3600 m a.s.l., which was situated just above the tree line and comprised high elevation shrubby grassland with some scattered trees and shrubs. The sites of 1000 m and lower are situated on alluvial and fluvial floodplains, while the geological substrate of the Kosñipata Valley consists of Palaeozoic sedimentary rocks ranging from Ordovician mudstones and slates to Permian granite. A summary of the mean annual temperatures (°C), mean annual soil temperatures (°C), and annual precipitation (mm $year^{-1}$) throughout the Kosñipata Valley can be found in Table2.

2.2.2 Leaf analysis

Between 3 May 2007 and 11 July 2007, four individual trees from four to five dominant species from each 1-ha plot were selected. From each tree, three to four fully expanded, non-damaged, sunlit leaves were collected for physical and chemical analysis. Directly after collection, leaf fresh weight was determined with a high precision (± 0.002 g) pocket scale (MyWeigh, Gempro 50, Taiwan) and leaf thickness was measured with high precision callipers (MW201-01 DAB, Bowers Metrology Ltd, UK) at five different points per leaf, avoiding the veins. Leaf area was determined by taking a digital image of a flattened fresh leaf (*e.g.* Grotkopp and Rejmanek 2007; Royer *et al.* 2009) and analysed for area using ImageJ (version 1.38x, NIH, USA).

Table 1. Field site number and with altitude, location, vegetation type, genera of the sampled species, geological substrate, and the percentage of *Fabaceae* present in the sampled species with the percentage of *Fabaceae* present in the 1-ha research plots in parentheses.

Field site	**Latitude**	**Longitude**	**Mean altitude a.s.l. (m)**	**Type of vegetation**	**Sampled species**	**Geological substrate**	**Percent *Fabaceae* trees in sample (in research plot)**
1	-12.83	-69.28	220	Tropical rain forest	*Iryanthera juruensis, Bixa arborea, Brosimum guianense, Micropholis guyanensis, Neea divaricata*	Alluvial deposits (old floodplain)	0 (7.25)
2	-12.83	-69.3	220	Tropical rain forest	*Leonia glycocarpa, Mabea nitida, Pourouma cecropiifolia, Rinoera viridifolia, Symphonica globulifera*	Fluvial deposits	0 (5.80)
3	-12.95	-71.53	1000	Tropical rain forest	*Ficus sanguinosa, Virola elongate cf, Pourouma minor, Pouteria sp.*	Alluvial deposits (old floodplain)	25 (9.66)
4	-13.05	-71.54	1500	Lower montane cloud forest	*Molinedia simulans, Tachigali setifera cf, Guatteria sp., Miconia sp.*	Palaeozoic sedimentary rock (granite)	0 (8.97)
5	-13.07	-71.55	1855	Lower montane cloud forest	*Cyathea lechleri, Hedyosmum racemosum, Clethra revoluta, Myrcia sp.*	Palaeozoic sedimentary rock (sandstone)	0 (0.16)
6	-13.17	-71.58	2350	Upper montane cloud forest	*Hedyosmum scabrum, Clusia sp., Miconia sp., Prunus sp.*	Palaeozoic sedimentary rock (mudstone)	0 (0)
7	-13.19	-71.59	2990	Upper montane cloud forest	*Clusia cretosa, Weinmania crassifolia, Schefflera allocotantha, Clethra cuneata*	Palaeozoic sedimentary rock (mudstone)	0 (0)
8	-13.2	-71.62	3600	Shrubby grassland	*Polylepis paulta, Gynoxis sp, Buddleja sp, Pluchea sp*	Palaeozoic sedimentary rock (sandstone)	0 *(No trees)*

Leaves were air-dried immediately, followed by drying to constant mass at 60 °C in the laboratory. Leaf mass per area (*LMA*, g m^{-2}) was calculated by dividing dry mass by the fresh leaf area, while leaf tissue density (L_d, g cm^{-3}) was determined after the

definition of Ryser (1996). This method assumes that leaf dry matter content (LDMC) is equal to L_d and is based on the tight relationship between volume and fresh weight of the leaf (Witkowski and Lamont 1991; Garnier and Laurent 1994). LDMC is often used to determine tissue density (*e.g.* Westoby 1998; Wilson *et al.* 1999) because of analytical simplicity. The method was compared with an alternative way to calculate L_d, which consisted of dividing the leaf dry mass with the volume (based on the measured thickness and area) of the leaf. No significant differences between these methods were observed in our study, so the method that assumes LDMC ~ L_d is used in this paper. Subsequently, the leaves were analysed individually for N and P concentrations and their total N and P content was calculated. Nitrogen concentrations were determined by using an elemental analyser (NA 2500 Carlo Erba Instruments, Milan, Italy); aspartic acid was used as an internal standard and cyclohexanaone standards for calibration. Phosphorous concentration was determined by using the molybdenum blue method (Grimshaw *et al.* 1989) using an auto analyser (Bran and Luebbe Auto Analyser III). The calculated *LMA* values were used to convert mass-based nutrient concentrations to concentrations on an area basis.

Table 2. Mean annual air temperature (ºC), soil temperature at 10 cm depth (ºC), and annual precipitation rates (mm $year^{-1}$) at different altitudes in the Kosñipata Valley, Peru. Means are based on continuous measurements at the sites from September 2006 – September 2008.

Elevation (m)	**Mean annual air temperature (°C)**	**Mean annual soil temperature (°C)**	**Annual precipitation (mm yr^{-1})**
220	26.4	23.9	2730
220	26.4	24.5	2730
1000	20.7	20.7	3087
1500	18.8	19.06	2631
1855	18.03	17.4	2472
2990	12.5	11.5	1706

2.2.3 Statistical analysis

Statistical tests were made in Minitab v. 15.1. To obtain a global impression of the relationship between the measured variables and altitude, linear regressions of the leaf traits and altitude were performed. Generalised linear models were used to test for the influence of site (altitude) and species as a nested factor within sites. To test for significant differences between sites and species, post hoc Tukey tests were performed. As none of the species within a site showed significant differences for all measured variables from the other species, the data from all species were pooled for the analyses between sites (for data on species, see supplementary material, table 1). To describe the bivariate relationships between the measured variables, each of which has an associated error, standard major axis regressions (Model II) were made on the log-transformed dataset (Wright, *et al.* 2004; Warton *et al.* 2006).

2.3 Results

2.3.1 Leaf mass per area (LMA) and leaf density (L_d)

The linear regression indicated for *LMA* a significant, almost-linear, increase with altitude ($R^2 = 0.93$, $P < 0.0001$) (Figure 1a). The sites at the two lowest altitudes had leaves with smaller *LMA* (64 ± 4.0 g m^{-2} and 72 ± 3.9 g m^{-2}), while leaves from the two highest altitudes had *LMA* values that were more than twice as large (152 ± 7.2 g m^{-2} and 175 ± 4.9 g m^{-2}). L_d on the other hand did not show a similar pattern; it differed significantly among sites, but there was no trend with altitude. Leaves with the largest L_d (up to 0.44 ± 0.008 g cm^{-3}, $P < 0.05$) were found at the lowest and highest altitude sites (220 and 3600 m a.s.l.), and at one site in the middle of the altitudinal transect (1500 m a.s.l.).

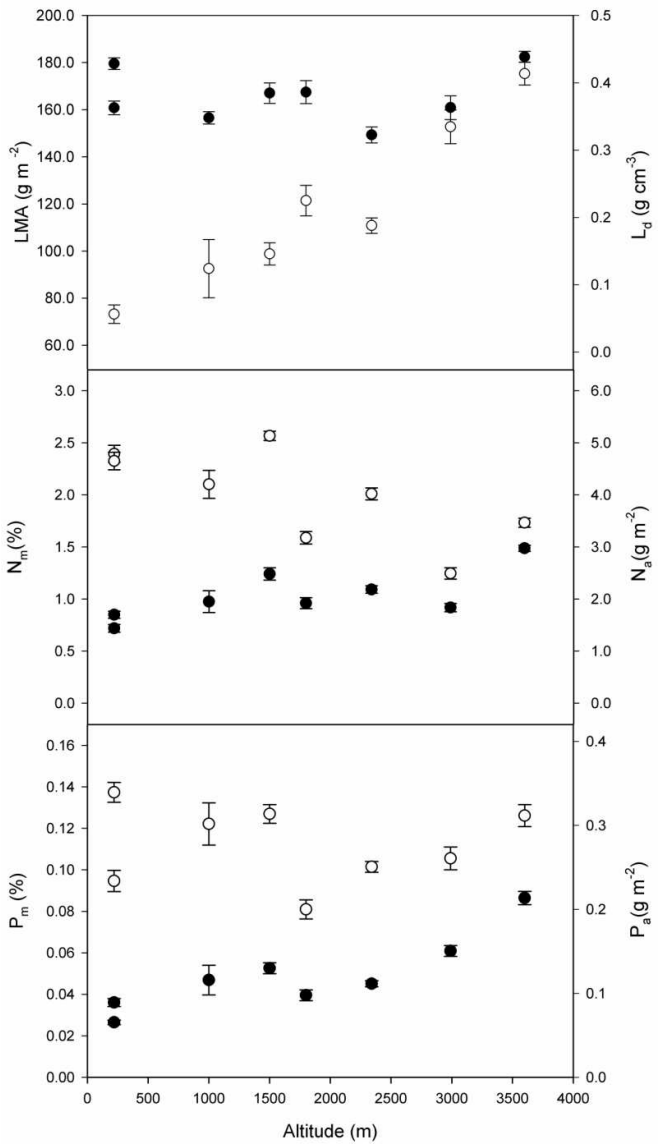

Figure 1. Leaf traits in the Kosñipata Valley, Peru for the most abundant species per altitude ± SE (sampled trees per plot = 12-15). **(A)** Leaf mass per area (*LMA*, g m^{-2}) and leaf density (L_d, g dry mass m^{-3}). White circles denote *LMA* and black L_d. The linear regression between altitude and *LMA* was highly significant ($R^2 = 0.93$, $p < 0.0001$), with a regression coefficient of 0.0299. No significant relationship was found between altitude and leaf tissue density. **(B)** Nitrogen content on a mass (N_m, %) and area (N_a, g m^{-2}) basis. White circles denote N_m and black circles denote N_a . The linear regression between altitude and N_m was significant, ($R^2 = 0.44$, $P< 0.05$), with a regression coefficient of - 0.00026. No significant relationship was found between altitude and N_a. **(C)** Phosphorus content on a mass (P_m, %) and area (P_a, g m^{-2}) basis. White circles denote P_m and black circles denote P_a. No significant relationship was found between altitude and P_m. The linear regression between altitude and P_a was significant ($R^2 = 0.704$, $P < 0.01$), with a regression coefficient of 0.000032.

2.3.2 Leaf nutrient contents

Relationships between elevation and foliar chemical composition were highly dependent on whether nutrient concentrations were expressed on a mass or area basis, and which nutrient concentration was being considered. A significant relationship was found between N_m and altitude ($R^2 = 0.44$, $P < 0.05$), with N_m being highest at the LRF sites (2.39 % ± 0.082) and lowest at the higher altitude sites (1.25 % ± 0.054). No significant relationship with altitude was observed for N_a (Figure 1b), although N_a was marginally higher at the highest elevation site than lower down. Significant differences were found for P_m amongst sites, however without a significant trend with altitude (Figure 1c). The two lowland sites (220 m a.s.l.) showed a substantial difference in values of P_m, (0.095 vs. 0.137 %, $P < 0.05$), even though they were less than 5 km apart. Finally, in contrast to the lack of systematic differences in N_a among high and low altitude sites (Figure 1b), P_a showed a significant increase with altitude, from 0.065 g m^{-2} ± 0.0027 at one of the 220 m a.s.l. sites to 0.213 g m^{-2} ± 0.0079 at 3600 m a.s.l. (Figure 1c). Like P_m, the P_a values at the two sites at 220 m a.s.l. differed significantly (0.065 vs. 0.089 g m^{-2}, $P < 0.05$). On a mass basis, the two highest elevation sites had the lowest N:P ratios (15 and 12), and whilst there were significant differences among sites, no significant trend with altitude was found (Figure 2).

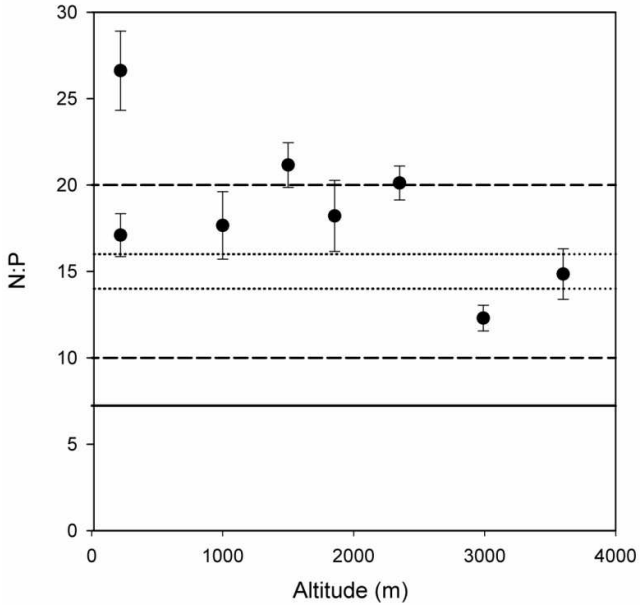

Figure 2. N:P ratios for most the abundant species per altitude ± SE (sampled trees per plot= 12-15). The solid black line represents the Redfield ratio on a mass basis (Redfield, 1958). The dotted lines represent the boundaries for N and P limitation according Aerts and Chapin (2000), with N:P <14 corresponding to N limitation and N:P > 16 to P limitation. The dashed lines represent the boundaries for N and P limitation (N:P < 10 and N:P> 20, respectively) according to Gusewell (2004). No significant relationship was found between altitude and N:P.

2.3.3 Relationships between N, P, LMA and L_d

The strongest positive bivariate leaf trait relationships were found between the log-transformed values of N_a and P_a (adj. $R^2 = 0.58$; Table 3), and between N_m and P_m (adj. $R^2 = 0.31$; Table 3). *LMA* exhibited negative significant relationships with both P_m and N_m, but these relationships were different in their coefficient of determination. The relationship between *LMA* and P_m was significant, though very noisy (adj. $R^2 = 0.016$, $P < 0.05$), compared to the adj. R^2 of 0.31between *LMA* and N_m. The bivariate log relationships between *LMA* and N_a or P_a were both positive and strong compared to the other relationships (adj. $R^2 = 0.25$ and 0.45; Table 3). Other statistically significant relationships between the parameters could be found as shown in Table 3, though their values of adj. R^2s were very low (< 0.14).

Table 3. The bivariate relationships between log-transformed measured leaf traits from the total dataset collected in the Andes-Amazon transect in the Kosñipata Valley, Peru. Leaf traits include: nitrogen and phosphorus on a mass (N_m and P_m, respectively) leaf mass per area (*LMA*), leaf density (L_d), and nitrogen to phosphorus ratio (N:P). Pre-log-transformed units of the leaf traits are as found in Figures 1 and 2. Standardised major axis (SMA) slopes and their 95% confidence intervals are given in the upper right section of the matrix (*x*–axis variables are given in the rows; *y*-axis variables are in the columns. The inverse of these slopes describe the regression coefficient from *y* on *x*). The adjusted R^2 values for significant bivariate relationships ($P < 0.05$) are given in the lower left section of the matrix (n = 304-320). The log-log relationships between N_m and N_a and P_m and P_a are not shown. NS, non-significant.

Log-transformed Parameters	N_m	P_m	*LMA*	L_d	N_a	P_a	N:P
N_m	-	0.99 (1.08, 0.90)	-0.68 (-0.61, -0.75)	NS	-	-0.69 (-0.61, -0.76)	0.53 (0.58, 0.47)
P_m	0.31	-	-0.61 (-.75, -0.68)	-1.24 (-1.11, -1.37)	0.92 (1.02, 0.82)	-	-0.53 (-0.47, -0.59)
LMA	0.31	0.016	-	-1.81 (-1.64, -1.98)	1.34 (1.46, 1.23)	1.01 (1.09, 0.94)	-0.78 (-0.70, -0.85)
L_d	NS	0.053	0.10	-	0.74 (0.82, 0.66)	-0.56 (-0.50, -0.62)	NS
N_a	-	0.028	0.25	0.10	-	0.75 (0.81, 0.70)	NS
P_a	0.031	-	0.45	0.017	0.58	-	-0.76 (-0.68, -0.85)
N:P	0.063	0.022	0.049	NS	NS	0.08	-

2.4 Discussion

2.4.1 Leaf mass per area and leaf tissue density

The leaves from our study showed an increase in *LMA* with altitude, as found elsewhere in both temperate (*e.g.* Körner 1989; Diemer *et al.* 1992; Pyankov *et al.* 1999) and tropical mountains (Vitousek *et al.* 1992; Kitayama and Aiba 2002; Moser *et al.* 2007). However, despite the similar trend of increasing *LMA* with altitude at these tropical sites, the absolute values of *LMA* in our study (Table 4) were up to 50% lower than those previously reported. This difference can be partially explained by the different sampling schemes among some of the studies listed in Table 4. Firstly, in the Hawaiian study (Vitousek *et al.* 1992), only one species was sampled along an altitudinal transect. Secondly, in the study in Ecuador (Moser *et al.* 2007), *LMA* was calculated from fresh litter samples, thus including leaves from all the layers of the canopy. Sun leaves usually have higher *LMA* values and as a consequence higher N_a values (Evans and Poorter 2001; Meir *et al.* 2002; Markesteijn *et al.* 2007; Meir *et al.* 2007; Niinemets 2007). This may explain some differences between studies, however, based on our sampling technique, we would have expected our *LMA* values to be higher than those found by Moser *et al.* (2007) in fresh litter, rather than lower. Our results also contrast with those of Kitayama and Aiba (2002), who reported *LMA* values of sunlit MCF leaves at similar altitudes in Borneo to be up to 50% higher than the *LMA* values observed in our study. Taken together, it seems likely that *LMA* values are indeed lower along the MCF altitudinal transect in Peru compared to the above cited studies.

Increases in *LMA* either reflect an increase in L_d or in leaf volume to area ratio (Poorter *et al.* 2009), both of which can differ within and among species (*e.g.* Witkowski and Lamont 1991). In our study, the bivariate relationship between *LMA* and L_d, though significant, was weak (adj. $R^2 = 0.10$), and negative; the observed increase in *LMA* with elevation (Figure 1a) is mostly a result of an increase in leaf volume to area ratio, rather than changes in L_d. We are not aware of other L_d data from a tropical elevation transect, but a study on L_d from conifer needles in the Alps showed no relationship between L_d altitude for one species, and a decrease in the

summer season with L_d for a second species (Birmann and Körner, this issue). Furthermore, other studies in temperate regions have shown that the number of leaf mesophyll layers and the cell wall thickness both increase with altitude (Körner *et al.* 1986; Körner and Diemer 1987; Körner 1989; Kogami *et al.* 2001).Edwards and Grubb (1982) suggested that the cell walls of MCF leaves might be thicker in order to protect them from fungal infections in this relatively cold and moist environment. Furthermore, the intercellular space and proportion of mesophyll exposed to the intercellular space have been shown to increase with altitude (Körner and Diemer 1987; Körner 1989) in order to adapt to the lower atmospheric pressure of CO_2 at higher altitudes. It might be possible (although we did not measure it) that a combination of thicker cell walls and more intercellular space balance each other out in our data, leaving no trend with altitude L_d.

2.4.2 Nutrients

Nitrogen

Consistent with other tropical elevation gradient studies (Grubb 1977, Proctor *et al.* 1989, Vitousek *et al.* 1992, Tanner *et al.* 1998, Kitayama and Aiba 2002, Soethe *et al.* 2008), the higher-elevation leaves had significantly lower N_m values (Table 4), although the overall pattern in our N_m with altitude was weak, perhaps because of the geographical spread in locations of the study plots; not all plots were located on the same part of the mountain ridge (Table 4; cf. Körner (2007). Nevertheless, a decrease in N_m was still observed and is weakly consistent with Tanner *et al.* (1998), who suggested that lower foliar N_m at higher altitudes in MCFs is caused by lower mineralization rates.

Aside from lower temperatures, mineralization rates at higher altitudes in MCFs can also be limited because of high water saturation of the soils or changes in the decomposer community. Many studies of soils in MCFs have reported persistent (near) water-saturated conditions in the soils (*e.g.* Grubb and Whitmore 1966, Grubb 1977, Bruijnzeel *et al.* 1993) and incubation studies have shown that by decreasing the water content of soils from MCFs, N mineralization rates increase (Marrs *et al.* 1988; Cavelier *et al.* 2000). Waterlogged soils have not been observed in our study

sites, so this phenomenon is unlikely to have influenced nutrient availability in this study. As for changes in decomposer communities, at higher altitudes in tropical gradients, lower densities of invertebrates are found (Olson 1994; Richardson *et al.* 2005; Illig *et al.* 2008) and especially litter feeding micro-arthropod taxa are rare in MCFs (Illig *et al.* 2005). Furthermore, specific leaf trait combinations can feed back on nutrient cycling, with low foliar nutrient concentrations and high *LMA* constraining decomposition and hence limiting nutrient availability (Cornwell *et al.* 2008).

N_m in this study (Table 4) was higher than found in most other tropical elevation gradient studies (Vitousek *et al.* 1992; Tanner *et al.* 1998; Kitayama and Aiba 2002), although similar values were observed by Soethe *et al.* (2008) in Ecuador. As with the differences in *LMA*, it is unlikely that methodological differences in leaf sampling are the principal explanation. Although leaf standards were not analysed alongside our leaf samples, the analyser was fully calibrated and we note that similar values have been obtained for trees on the same transect using an entirely independent method (Kjeldahl; J.B.Fisher pers. comm.); we thus consider our relatively high N_m values to be real. Since mean annual temperatures (Table 2) at this site do not vary by more than 1.5 °C from the other MCF studies listed in Table 4, it is unlikely that differences in temperature and hence mineralization influenced N-cycling in an unusual way in our transect. The selected species in our study are also an improbable cause for the overall higher N_m values. Townsend *et al.* (2007) showed how foliar N_m of neotropical forests was almost 40% higher in leaves derived from plants from the Fabaceae family. Only the pooled sample from site 4 (1500 m a.s.l.) included a species of this family - which could explain the higher N values at that particular site - but Fabaceae species were not in the other samples. Although Fabaceae were present at the lower altitude sites, they were not sampled in this study (Table 1), and no N-fixing species occurred at the four highest-elevation plots. Hence, the presence of Fabaceae species in our data set is an unlikely explanation for finding relatively high foliar N values across all sample sites.
The combination of a large N_m and small *LMA* (Figure 3) is consistent with a global survey of leaf traits (Wright *et al.* 2004). The consequence of larger *LMA* at higher

altitudes is that the observed decrease in N_m with altitude was reversed for N_a in our data, as also observed in MCFs in Borneo and Hawaii (Proctor *et al.* 1989; Vitousek *et al.* 1992; Kitayama and Aiba 2002), but not in a MCF in Ecuador, where both N_a and N_m declined with altitude (Soethe *et al.* 2008).

Table 4. Maximum and minimum foliar nitrogen (N_m) and phosphorus (P_m, leaf mass per area (*LMA*, g m^{-2}) and N:P ratio values reported from literature with comparable tropical altitudinal transects, together with the altitudinal ranges of these studies.

Publication	**Location**	**Altitudes of transect minimum and maximum (m)**	**N % (max-min)**	**P% (max-min)**	**N:P ratio on mass basis (max-min)**	***LMA* (g m^{-2}) (max-min)**
This study	Peru	220-3600	2.39-1.25	0.137-0.081	25-12	169-63
Kitamaya and Aiba (2002)	Borneo (Mt Kinabalu, ultramafic soil)	700-3050	1.06-0.49	0.011-0.008	96-36	215-120
Kitamaya and Aiba (2002)	Borneo (Mt Kinabalu, sedimentary soil)	650-3080	1.47-0.72	0.026-0.019	61-29	290-145
Vitousek *et al.*(1992)	Hawaii, old soil	250-2400	1.12-0.54	0.076-0.037	19-13	460-90
Soethe *et al.* (2008)	Ecuador	1900-3000	2.2 -1.13	0.140-0.022	10-8.1	
Moser *et al.* (2007)	Ecuador	1900-3000				190-114
Grubb (1977)	New Guinea	80-3700	2.08-0.98	0.146-0.055	18-14	
Grubb (1977)	Malaya	90-1800	1.55 -1.00	0.071-0.042	24-22	
Medina *et al.* (1981)	Puerto Rico	500-1050	1.36-0.99	0.054-0.063	25-16	
Proctor *et al.* (1989)	Borneo (Mt Silam)	330-870	1.72-1.34	0.046-0.054	32-29	

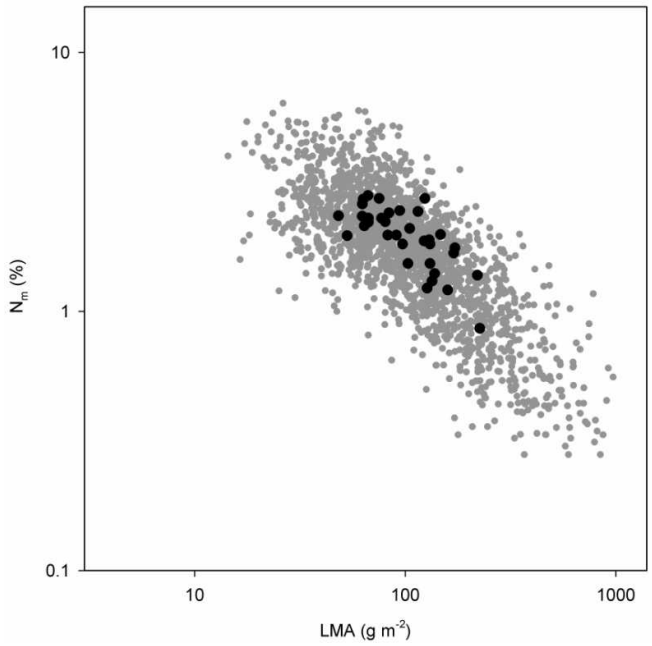

Figure 3. *LMA* (g m^{-2}) and N_m (%) values of the species from our study in the Kosñipata Valley, Peru (black circles), together with the GLOPNET data set from Wright *et al.* (2004), covering the leaf economic spectrum. Note the logarithmic scale.

A possible explanation for the higher values of foliar N observed in this study compared with other MCFs is the that N deposition rates in the eastern Andes, influenced by biomass burning in lowland Amazonia, appear to be large (Fabian *et al.* 2005; Boy *et al.* 2008). Boy *et al.* (2008) showed that during the burning season in Amazonia, N deposition rates in north Ecuador were double that in non-fire seasons, leading to an annual deposition rate of 5.1-5.4 kg ha^{-1} year^{-1}, which is substantially larger than the global average of 3.5 kg ha^{-1} year^{-1} (Phoenix *et al.* 2006). Fabian *et al.* (2005) also reported high N deposition rates following Amazonian biomass burning for a site in Ecuador showing similar N_m values to ours (Table 4; Soethe *et al.* 2008). Nitrogen deposition rates are not available for Peru. However, since our research sites also receive air and moisture fluxes from north and west Amazonia (Marengo *et al.* 2004; Killeen *et al.* 2007), higher N deposition as a

consequence of Amazonian biomass burning could help explain why the N_m values in our study were relatively high (Table 4).

Phosphorus

There was no clear relationship between P_m and altitude in our data, and furthermore, inter-site differences in P_m were also large at the lowland sites, even though their N_m values did not differ significantly. The lack of trend with altitude in P_m is inconsistent with most other tropical elevation studies cited in Table 4. Most showed decreases in P_m with altitude (Grubb 1977; Vitousek *et al.* 1992,; Tanner *et al.* 1998; Soethe *et al.* 2008), although Kitayama and Aiba (2002) similarly failed to identify significant trends in Borneo. P_a did show an increasing trend with altitude, though this was a consequence of the increase of *LMA* with elevation (Figure 1a).

Several factors influence the availability of P along tropical altitudinal gradients. Firstly, like N, the availability of P is expected to decrease with altitude as a result of slower decomposition and nutrient cycling at lower temperatures. However, unlike N, P does not enter terrestrial ecosystems in a biologically available form through microbial reactions. Instead, nearly all active P is derived from mineral weathering (Smeck 1985). Generally, in the first stage of soil development, P is present in a mineral form, and then becomes biologically available through weathering during the beginning of soil development. Finally, P becomes less available through leaching and conversion to recalcitrant forms by binding to iron (Fe) and aluminium (Al) oxides and hydroxides rendering it difficult for biological uptake (Walker and Syers 1976; Crews *et al.* 1995). Indeed, tropical soils of intermediate age have higher P availability than old, undisturbed soils (*e.g.* Pearson and Vitousek 2002; Porder *et al.* 2007). Therefore, while reduced P-cycling as a consequence of lower temperatures would decrease P_m, we expected that the younger, more rapidly eroding soils at higher altitudes would have led to relatively high P availability. Furthermore, since the research sites at higher altitudes experience lower temperatures (Table 2), plants at higher altitudes might accumulate P in order to maintain their photosynthesis at low temperatures, since low temperatures reduce phosphate recycling (Savitch *et al.* 1997; Strand *et al.* 1997; Sage and Kubien 2007). The combination of higher P availability and requirement would therefore lead to higher foliar P levels.

One explanation for the lack of altitudinal trend is that disturbances such as landslides, which frequently occur throughout the Kosñipata valley (Feeney 2004), have influenced the site specific histories and soil age, and thus potentially leaving a heterogeneous pattern of P availability with altitude. The difference in substrate (fluvial vs. alluvial deposits) can also explain the large difference in P_m in the lowland sites, with the fluvial deposits having a higher P availability due to more recent flooding events (Table 1). In addition, other processes that were not studied could have influenced foliar P content. For example, some studies have shown better nutrient resorption for N than for P, depending on the species (*e.g.* Aerts 1999; Martinez-Sanchez 2003) and patterns of N:P ratios show higher N:P ratios for tropical leaf litter than fresh foliage, indicating that tropical species have higher P resorption capacities (McGroddy *et al.* 2004). If MCF species resorb P more strongly than N before leaf abscission, P availability could be less affected by decreased decomposition at higher altitudes. Whilst these explanations remain speculative, the data presented here (Figure 2) suggest that not all tropical altitudinal transects show simple trends in P_m with elevation.

2.4.3 Ecological stoichiometry and nutrient limitations

Though both N_m and P_m are discussed as separate variables above, the ratio between N and P is also of ecological interest (Sterner and Elser 2002). The standardised major axis regression coefficients for N_m and P_m, and N_a and P_a, were positive and had the highest adj. R^2 values (Table 3). Thus, higher N and P contents generally co-occur, even though the patterns of N and P (both on an area and mass basis) vary separately with altitude. Table 4 shows that although N_m and P_m at our study sites are mostly higher than in studies in other MCFs, the observed N:P ratios are within the same range, except for the studies based on Mt Kinabalu in Borneo (Kitamaya and Aiba, 2002), which reported much higher N:P ratios caused by the low foliar P_m values there. It is therefore possible, that the large foliar N_m in our transect is a stoichiometrical consequence of the trees taking up more N because of a relatively high availability of P in our study sites (Sterner and Elser 2002).

Though it has to be kept in mind that true nutrient limitation can only be shown through experimental manipulations as shown in studies such as Tanner *et al.* (1992) and Vitousek *et al.* (1995), N:P ratios provide useful indications whether plants might be N or P limited. Generally, the 'Redfield ratio', a molar N:P ratio of 16:1 (7.23 on a mass basis), is assumed to be the optimum ratio of the two nutrients for an autotrophic organism to function (Redfield 1958). All our study sites showed N:P ratios higher than the Redfield ratio (Fig. 2). However, the Redfield ratio is derived from marine algae studies, and may not be applicable to more complex autotrophic organisms that require different N:P ratios for different tissue types (Sterner and Elser 2002; Kerkhoff, *et al.* 2005; Ågren 2008). Indeed, for terrestrial autotrophs such as trees, N:P ratios are much more variable throughout biomes and amongst and within species, and although analogous biogeochemical constraints may exist for individual plant organs, differences can vary by up to an order of magnitude (Sterner and Elser 2002; Gusewell 2004; McGroddy, *et al.* 2004). None the less, average N:P values might serve as a useful indication for nutrient limitation.

According to Aerts and Chapin (2000), a mass basis N:P ratio lower than 14:1 indicates N limitation for plants, while a ratio higher than 16:1 suggests a P deficiency. Most of the N:P ratios we found were higher than 14, except for the study site at 2990 m a.s.l. This suggests that except for the leaves at 2990 m a.s.l., none of the research sites were N limited. In addition, many of the sites showed N:P ratios higher than 16. This also holds true for many of the studies cited in Table 4, which only shows a few sites with N:P ratios lower than 14, such as the sites in Ecuador (Soethe *et al.* 2008). Gusewell (2004) suggested a broader range of N:P ratios to determine N or P limitation, with N:P < 10 indicating N limitation and N:P > 20 corresponding to P limitation. If we apply these ratios to our dataset, none of the research sites are limited by N, and only some of the lowest altitudes by P. In the absence of evidence from experimental manipulations in this study, the higher N:P ratios at lower elevations presented here provide indirect evidence that, consistent with other studies (*e.g.* McGroddy, *et al.* 2004; Lovelock *et al.* 2007; Meir, *et al.* 2007; Townsend, *et al.* 2007; Reich, *et al.* 2009), though not all (*e.g.* Mirmanto *et al.* 1999), tropical forests are P-limited at low altitude. However, contrary to the general hypothesis suggested by Tanner *et al.* (1998), the relatively high N:P ratios observed

at high elevation in our study, suggest that N limitation may not occur in southern Andean MCFs. If this is true, the impact on tree growth of possible future increases in N deposition (Phoenix *et al.* 2006) may be slight.

2.5 Conclusions

We describe trends with altitude for rain forest in south-east Peru of increasing *LMA* and decreasing N_m that are similar to those found for other MCFs. However the absolute values reported here, and from other MCFs in the tropical Andes, appear to be larger for N_m and lower for *LMA* than those found for MCFs in Central America and south-east Asia, although they all are within the range of values reported recently for a global dataset for which MCF data were absent (Wright *et al.* 2004). P_m did not vary significantly with altitude along this transect, and instead we note high spatial variability in P_m, especially at low elevations. Leaves at high altitude had smaller N:P ratios than those at low altitude, but showed little evidence of N-limitation (Guswell 2004; cf. Tanner *et al.* 1998). Taken together with the relatively high absolute values of N_m, these results suggest that the hypothesis of N limitation in MCFs may not apply in the south-east Andes.

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Chapter 3

Paper 2: Photosynthetic parameters, dark respiration and leaf traits in the canopy of a Peruvian tropical montane cloud forest

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Abstract

Few data are available describing the photosynthetic parameters of tropical montane cloud forests (TMCF) and their relationship to other leaf traits. Here, we present a study of photosynthetic leaf traits (V_{cmax} and J_{max}), foliar dark respiration (R_d), foliar nitrogen (N) and phosphorus (P) and leaf mass per area (*LMA*) throughout the canopy for five different TMCF species at 2990 m a.s.l. in Andean Peru. TMCF V_{cmax} and J_{max} on a leaf area basis were similar to values found in lowland tropical rainforest leaves when corrected to 25 °C (55.6 ± SE 2.6 and 106.5 ± SE 5.2 μmol m^{-2} s^{-1}, for V_{cmax} and J_{max}, respectively), but much lower when standardized to TMCF ambient mean temperature, compared with ambient lowland tropical rainforest values. Dark respiration was relatively high (1.43 ± SE 0.06 μmol m^{-2} s^{-1}) when standardised to 25 °C and similar to ambient tropical lowland values when corrected to 12.5 °C. The TMCF J_{max}-V_{cmax} relationship was steeper than found in other tropical biomes, and resembled those found in tropical understorey plants. V_{cmax} showed a significant relationship with N, but not with P on an area basis, though the fit with N was relatively weak ($P<0.05$, $R^2=0.06$, n = 78). The V_{cmax}–N relationship (*i.e.* nitrogen use efficiency) combined with the $V_{cmax\text{-}area}$ observed here showed that neither V_{cmax}-N relationships from tropical nor temperate regions adequately predict TMCF V_{cmax} and as consequence, TMCFs qualify as a separate plant functional type.

Keywords: Tropical montane cloud forests, leaf mass per area, foliar nutrients, photosynthesis, dark respiration, A-C_i curves.

3.1 Introduction

Tropical montane cloud forests (TMCF) can be found in tropical mountainous areas, usually between 1000 to 3000 m above sea level (a.s.l.). A distinguishing characteristic of these forests is the frequent occurrence of clouds and mist at canopy or ground level (Stadtmüller 1987, Hamilton *et al.* 1995). Over recent years, different components of the carbon (C) cycle in tropical montane cloud forests (TMCF) have been studied, including: soil and root respiration and soil C stocks (Schuur and

Matson 2001, Iost *et al.* 2008, Zimmermann *et al.* 2009a, Zimmermann *et al.* 2009b), and the above- and belowground TMCF productivity as determined through changes in stem growth, litter fall, root growth and leaf are index (LAI) (*e.g.* Grubb and Whitmore 1966, Veneklaas 1991, Bruijnzeel and Veneklaas 1998, Roderstein *et al.* 2005, Leuschner *et al.* 2007, Moser *et al.* 2007). Overall, aboveground net primary productivity (ANPP) in TMCFs is lower than in tropical lowland forests, which has been attributed to lower mean annual temperatures, reduced levels of photosynthetically active radiation (PAR) through frequent cloud events, leaf wetness, and low nutrient status of the leaves (Bruijnzeel and Veneklaas 1998, Waide *et al.* 1998, Letts and Mulligan 2005). However, photosynthesis, the process that underpins the gross C-input in an ecosystem, is relatively little studied in TMCFs (Hikosaka *et al.* 2002, Letts and Mulligan 2005). In general, the controls on canopy C-uptake can be divided into: (i) environmental controls (*e.g.* temperature, VPD, radiation quantity and quality); (ii) canopy structure (LAI and leaf angles); and (iii) natural variation in the photosynthetic capacity of canopy leaves, which, together with the rate of dark respiration forms the main focus of this study.

Maximum CO_2 assimilation rate at saturating photon flux density (A_{sat}) is reported in TMCFs to range from 3.5 to 10.2 μmol m^{-2} s^{-1} in Ecuador and Colombia between 1450 and 2150 m a.s.l. (Letts and Mulligan 2005) and from 5.4 to 3.1 μmol m^{-2} s^{-1} in Malaysian Borneo between 600 and 3700 m a.s.l. (Hikosaka *et al.* 2002). However, these values were not standardised for temperature, making them hard to compare with each other or with values from other tropical biomes. Moreover, since A_{sat} is dependent on stomatal conductance (g_s) and mesophyll conductance (g_m), it is more appropriate to use both the maximum carboxylation velocity of the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (V_{cmax}) and the maximum rate of electron transport (J_{max}) when investigating a plant's photosynthetic capacity. In addition, unlike A_{sat}, the biochemical parameters V_{cmax} and J_{max} can be applied in fine scale and global productivity models that incorporate well tested Farquhar equation for leaf-level C_3 photosynthesis (Farquhar and Von Caemmerer 1982). In fact, quantifying V_{cmax} and J_{max} more accurately has shown to reduce the error in the results of global terrestrial biosphere models (GTM) as well as finer scale models

(*e.g.* Knorr and Heimann 2001, Zaehle *et al.* 2005, Alton *et al.* 2007, Kattge *et al.* 2009, Mercado *et al.* 2009).

It is well known that the foliar CO_2 exchange parameters A_{sat} (and hence V_{cmax} and J_{max}) and foliar dark respiration (R_d) are related to the foliar nitrogen (N) content, mainly because of the high investment of N in the photosynthetic apparatus (Field and Mooney 1986, Evans 1989), and high involvement of N-rich proteins in maintenance respiration for protein turnover (Penning De Vries 1975). Furthermore, P relates to both A_{sat} and R_d through the synthesis of new proteins, plasma membranes and nucleic acids (RNA and DNA), the recycling of ribulose-1,5-biphosphate, (RuBP) and ATP/ADP transformations (Sterner and Elser 2002). Additionally, leaf mass per area (*LMA*) is strongly correlated with foliar CO_2 exchange and foliar N and P content. High *LMA* values generally occur in leaves with a high longevity, low nutrient contents and hence low A_{sat} and R_d. These relationships have been confirmed by extensive analyses of global datasets which show relationships with A_{sat}, R_d , N ,P, and *LMA* (Reich *et al.* 1992, Reich *et al.* 1994, Reich *et al.* 1998, Wright *et al.* 2004, Reich *et al.* 2008, Poorter *et al.* 2009).

Field studies have shown that the parameters V_{cmax} and J_{max} , which underpin A_{sat}, correlate with N as well (Carswell *et al.* 2000, Meir *et al.* 2002, Coste *et al.* 2005, Kattge *et al.* 2009), which is why leaf N content is used for scaling V_{cmax} (and J_{max}) in many terrestrial biosphere models (*e.g.* Woodward *et al.* 1995, Sitch *et al.* 2003, Friend *et al.* 2009). However, in tropical regions, stronger correlations between foliar P and V_{cmax} and J_{max} than with N have been found (Raaijmakers *et al.* 1995, Meir *et al.* 2007) and Domingues *et al.* (2010) showed that V_{cmax} and J_{max} estimations from foliar N and P for a variety of tropical species improved by including *LMA* in their model. Overall, V_{cmax} and J_{max} for tropical regions area scarce, (Xu *et al.* 2009), and models using the current V_{cmax} estimates for upscaling GPP perform not as well for tropical forests, as they do for temperate regions (Kattge *et al.* 2009).

For TMCFs it is unclear how the photosynthetic parameters relate to foliar N and P and *LMA*, though they have been hypothesized to be limited in their productivity by

N, rather than P (Tanner *et al.* 1998). This study aims to provide a first quantification of TMCF V_{cmax} and J_{max} and investigates how the foliar CO_2 exchange parameters V_{cmax}, J_{max} and R_d scale with foliar N and P and *LMA*, both on a mass and area basis. Furthermore, because most previous studies on TMCF foliage only have included fully sunlit leaves, this study gives a first description on how the measured leaf parameters (V_{cmax}, J_{max}, *R*d, foliar N and P and *LMA*) are distributed throughout the TMCF canopy. Extra attention was paid to whether the measured TMCF species differed in their parameter-canopy relationships, by using a three level random regression model. This regression model attributes the observed variance of the dataset to different levels of the dataset, with the levels being canopy height, tree-to-tree variation (within one species) and variations between overall mean values per species.

3.2 Methods

3.2.1 Fieldwork site

The study was performed in a previously-established 1 ha plot in a TMCF located in the Kosñipata valley in south-eastern Peru at 2990 m a.s.l. (13º11'28''S / 71º35'24''W), near the Wayquecha research station (Silman *et al.*, in press). The research site has an easterly aspect, with a 28 % slope, a mean daily temperature of 12.5 ± SD 1.05 ºC and maximum air temperature of 21.2 ºC while mean daily VPD is 0.14 ± SD 0.1 kPa and mean annual rainfall is 1706 mm yr^{-1}. A 'wet season' can be distinguished from October until March with 46% of the rain falling from December to February. The forest at the research site is dominated by species of the *Weinmannia* and *Clusia* genera. The average top canopy height is 12.8 ± SD 4.4 m, with 10.9 m being the average centre of canopy height, and the leaf area index (LAI) is 4.15 ± SD 0.59. Measurements were carried out during the dry season in 2008, between 11 June and 1 July for 4 species, and between 12-14 August for the species *Clusia cretosa*.

3.2.2 Gas exchange measurements

For the gas exchange measurements, five species that together represent ~ 25% of the basal area in the research plot were selected. The measured species were *Weinmannia bangii*, *Clethra cuneata*, *Schefflera allocotantha*, *Clusia creatosa* and *Prunus integrifolia*. From these species, 5-6 individual trees were selected and for each tree CO_2 response curves (A-C_i curves) on leaves from the top (fully sunlit), middle and bottom part of the canopy were carried out, leading to a total of 82 A-C_i curves. Portable photosynthesis equipment (Li-Cor 6400, Li-Cor Inc, Lincoln, USA), fitted with an LED light source (6400-02B Red/Blue Light Source, Li-Cor, Inc, Lincoln, USA), was used to carry out the A-C_i curves, following the procedural guidelines in Long and Bernaccchi (2003) with CO_2 concentrations inside the chamber ranging from 50 to 2000 ppm. In order to get access to the canopy, branches were detached and immediately re-cut under water in order to reconstitute the water column (Domingues *et al.* 2010). After detachment, A-C_i curves were completed within 30-60 minutes. According to Santiago and Mulkey (2003), alterations in stomatal conductance during this process do not affect the calculation of V_{cmax}, unless stomatal conductance g_s declines to very low levels. In cases of very low g_s, measurements were excluded from the dataset. A further check on data quality used elsewhere (Kattge *et al.* 2009) was also applied: where $A_{sat}/N_{area} < 2$ μmol CO_2 g N^{-1} s^{-1}, data was excluded (~ 5 % of the leaves), leading to an ultimate dataset of 78 A-C_i curves.

Prior to conducting an A-C_i curve, tests were carried out to ensure the measurements were carried out at light-satured levels of photosynthetic active radiation (PAR) flux density. The PAR flux densities that were used differed amongst the canopy positions, and on average a PAR flux density of 1200 μmol m^{-2} s^{-1} was used for top canopy leaves, 700 μmol m^{-2} s^{-1} for middle canopy leaves and 500 μmol m^{-2} s^{-1} for lower canopy leaves. Values higher than these photon flux densities led to decreases in photosynthesis throughout the measurement of A-C_i curve, probably caused by photodamage. During the measurements, leaf chamber temperature was controlled to be 20 ºC, unless ambient temperatures were too low or high to ensure stable chamber temperatures. Average leaf temperature for the whole dataset was 20.43 ± SE 0.26

ºC. Relative humidity was maintained at ambient levels, but never over 80%. Following the A-C_i curves, R_d was measured after keeping the leaf in darkness for 20 minutes or longer in order to avoid transient changes in CO_2 release associated with post-illumination changes in metabolism (Azcón-Bieto and Osmond 1983). R_d measurements were standardised to a leaf temperature of 25 ºC using a global average of Q_{10} = 2.13 (Tjoelker *et al.* 2001). This global Q_{10} value was chosen because it is unknown whether TMCF Q_{10} resembles either a tropical or temperate value.

3.2.3 A-C_i response curve analysis

A curve fitting routine similar to Domingues *et al.* (2010) was used to analyse the A-C_i curves to calculate V_{cmax} and J_{max} on a leaf area basis ($V_{cmax\text{-}area}$ and $J_{max\text{-}area}$, respectively). The curve fitting is based on minimum least-squares and was developed for use in an "R" environment (R Development Core Team 2008). (See Appendix II of this thesis for the curve fitting code). The fits were obtained using the Farquhar biochemical model of leaf photosynthesis (Farquhar *et al.* 1980, von Caemmerer and Quick 2000), with a modification for triose phosphate utilization (TPU) by Harley *et al.* (1992). The enzymatic kinetic constants were taken from von Caemmerer (2000), assuming an infinite internal conductance term. A curve-fitting strategy was chosen to avoid the co-limited region of the A-C_i response curve: $V_{cmax\text{-}area}$ was only fitted to values of intercellular CO_2 concentrations (C_i) lower than 30 Pa, whilst $J_{max\text{-}area}$ was only fitted to C_i values higher than 45 Pa. The fitted parameters were scaled to a reference temperature of 25 ºC using temperature dependencies provided by Bernacchi *et al.* (2003), since this is the most common reference temperature used in the literature, and to provide a basis for comparison with existing data on $V_{cmax\text{-}area}$ (*e.g.* Wullschleger 1993, Kattge *et al.* 2009). Additionally, the same temperature dependencies were used to scale V_{cmax} and J_{max} to the mean annual temperature of the study site (12.5 ºC) to estimate the ambient values of the photosynthetic parameters.

3.2.4 Nutrient and LMA analysis

After the gas exchange measurements, the individual leaves were detached and a digital image of the flattened fresh leaf was made. Leaf area was later determined by analysing the image with image processing software ImageJ (version 1.38x, NIH, USA). After taking the digital image, the leaves were put in a re-sealable plastic bag containing silica gel of 28-200 mesh size with a minimum ratio of 10:1 of silica gel to fresh weight leaf material, in order to dry and hence preserve the leaf. Chase and Hills (1991) showed that with this method, leaves dry sufficiently overnight (12 hours) in order to prevent degradation. After each field visit, leaves were dried to a constant mass at 60 °C in the lab and weighed. Subsequently, the leaves were analysed individually for total N and P content. C and N concentrations were determined using an elemental analyser (NA 2500 Carlo Erba Instruments, Milan, Italy) against common standards, while P content was determined by using a modified Kjehldahl method (Bran & Luebbe Auto Analyser III). The leaf mass per area (*LMA*) was calculated by combining the leaf area and leaf dry weight measurement. *LMA* values were used to convert mass-based nutrient concentrations to concentrations on an area basis, and area based V_{cmax} and J_{max} to a mass basis.

3.2.5 Statistics

To describe the bivariate relationships between the measured variables on both an area and mass basis, each of which has an associated error, standard major axis regressions (SMA, or Model II) were made in Minitab v. 15.1.1 with the log-transformed dataset (Wright *et al.* 2004, Warton *et al.* 2006). In addition, for $V_{cmax\text{-}area}$-N_{area} relationships standard linear (Model I) regressions were performed, in order to compare the $V_{cmax\text{-}area}$-N_{area} data with values reported in the literature. The variation of the leaf traits with canopy height, incorporating the variance of this relationship between different trees and species, was assessed with a three level modelling technique (Snijders and Bosker 1999). All analyses were undertaken with the *MLwinN* software package (Version 2.10 Rabash *et al.* 2004). In principal, this method involves linear regression of the leaf traits against height in the canopy. Since many leaf traits are associated with average levels of irradiance, which tends to

have an exponential extinction coefficient with depth in a canopy, we natural-log transformed the leaf trait data before carrying out the regression. The three level random coefficient model generates at level 1 the following model:

$$\pi_{lts} = \beta_{0ts} + \beta_{1lts}\, h + e_{lts}$$

with π_{lts} representing the leaf parameters (measured at leaf "l", within tree "t", belonging to species "s"), β_{0ts} the intercept term which is allowed to vary between individual trees and species, $\beta 1_{lts}$ the coefficient term describing how π_{lts} varies with the sampling height *h* per tree and species and e_{lts} is the residual error term. At level two of the model, the intercept β_{0ts} can be described as

$$\beta_{0ts} = \beta_{00s} + r_{0ts}$$

with β_{00s} being the tree-level effect per species on the random intercept, and r_{0ts} the tree specific deviation, and β_{1lts} is described as:

$$\beta_{1lts} = \beta_{10s} + r_{1ts}$$

with β_{10s} explaining the tree level effect on the random slope and r_{1ts} being the tree specific deviation of the random slope. At level three of the model, β_{00s} is described as:

$$\beta_{00s} = y_{000} + u_{00s}$$

with y_{000} giving the common value of the intercept for each leaf of each tree and for each species, and u_{00s} the species specific variation around this value. β_{10s} is described as:

$$\beta_{10s} = y_{100} + u_{10s}$$

With y_{100} being the common value for the random slope for all leaves from all trees and all species, and u_{10s} describing the species-specific deviation from the slope. Combining the equations above, the model can be written as:

$$\pi_{lts} = y_{000} + r_{0ts} + u_{00s} + (y_{100} + r_{1ts} + u_{10s})\, h + e_{lts}$$

The 'null model' of this equation is therefore:

$$\pi_{lts} = \beta_{0ts} + e_{lts} \text{ or}$$
$$\pi_{lts} = y_{000} + r_{0ts} + u_{00s} + e_{lts}$$

The residual terms of the null model can be used to examine the inherent sources of variation in the untransformed dataset with:

$$\text{Var}(e_{lts}) = \sigma^2, \text{ var}(r_{0ts}) = \tau^2 \text{ and var}(u_{00s}) = \phi^2$$

The total variance in the data set is then $\sigma^2 + \tau^2 + \phi^2$. We can use the variance partition coefficient (*e.g.* $\sigma^2/(\sigma^2 + \tau^2 + \phi^2)$) to examine how much of the variance can be attributed to inter-tree differences, between-tree differences and in-between species differences. It has to be noted that when a large portion of the variance can be attributed to inter-tree differences, this does not necessarily mean a significant relationship of that leaf variable with height.

To test whether the random regression coefficient β_{1lts} is significantly different from 0, we compared the difference of -2loglikelihood (-2LL) values of both models with a chi-squared distribution with three degrees of freedom (since three variables were added to the model). In a similar fashion, we tested whether adding level 2 (r_{1ts}) and 3 (u_{10s}) to the random slope model provided a better fitting model that a singular slope, by comparing the -2LL values of the fits of these models with a chi-squared distribution (*df* = 1 and *df*=2 respectively). Because of the heteroscedastic nature of the random coefficient model, we centred the model around the average height of the canopy (10.9 m) in our dataset (Snijders and Bosker 1999).

3.3 Results

3.3.1 CO_2 exchange parameters

The average V_{cmax} values from the data set (species and canopy position pooled) were 55.6 ± SE 2.6 μmol m^{-2} s^{-1} when expressed on an area basis ($V_{cmax\text{-}area}$) and 389.7 ± SE 18.4 nmol g^{-1} s^{-1} on a mass basis ($V_{cmax\text{-}mass}$), when standardised to 25 ºC. When corrected to the mean annual temperature (12.5 °C), $V_{cmax\text{-}area}$ was 17.45 ± SE 0.82 μmol m^{-2} s^{-1}, while $V_{cmax\text{-}mass}$ was 246.2 ± SE 11.7 nmol g^{-1} s^{-1}. Average $V_{cmax\text{-}area}$ almost halved when descending from the top of the canopy (Figure 1). The positive relationship with height and $V_{cmax\text{-}area}$ was significant, and improved significantly when the regression coefficient (β1) was allowed to vary between species and trees ($P<0.05$, Table 1). For $V_{cmax\text{-}mass}$ no clear decline with canopy height could be observed (Figure 2 and Table 1). However, $V_{cmax\text{-}mass}$ varied quite widely among species, ranging from 130-839 nmol g^{-1} s^{-1}, and almost half of the observed variation could be attributed to inter-species differences (Figure 4). J_{max} per leaf area ($J_{max\text{-}area}$) and per leaf mass ($J_{max\text{-}mass}$) showed a similar pattern, with average values of 106.5 ± SE 5.3 μmol m^{-2} s^{-1} and 763.3 ± SE 41.3 nmol g^{-1} s^{-1}, respectively (Figure 1 and 2) when standardised to 25 °C. Standardised to 12.5 °C, $J_{max\text{-}area}$ was 50.51 ± SE 2.5 μmol m^{-2} s^{-1}, while $J_{max\text{-}mass}$ was 564.3 ± SE 11.7 nmol g^{-1} s^{-1}. Like $V_{cmax\text{-}area}$, $J_{max\text{-}area}$ had a positive significant relationship with canopy height and the fit of the multilevel regression model significantly improved when allowing β1 to vary between species and trees ($P<0.05$, Table 1). $J_{max\text{-}mass}$ did not show a significant relationship with canopy height (Figure 2, Table 1). A type II regression between both $V_{cmax\text{-}area}$ and $J_{max\text{-}area}$ suggested a fit of $J_{max\text{-}area} = V_{cmax\text{-}area} * 2.03 – 6.45$ ($R^2 =0.78$, $P<0.001$, $n = 78$) and the 95% confidence interval from the coefficient of this relationship was 1.88-2.27. Since a Type I analysis is the way most previous studies have reported the relationship between $V_{cmax\text{-}area}$ and $J_{max\text{-}area}$, this analysis was also performed and the relationship was $J_{max}= V_{cmax} * 1.80 + 6.35$ ($R^2 =0.78$, $P<0.001$, $n = 78$); the 95% confidence interval from the coefficient of this relationship was

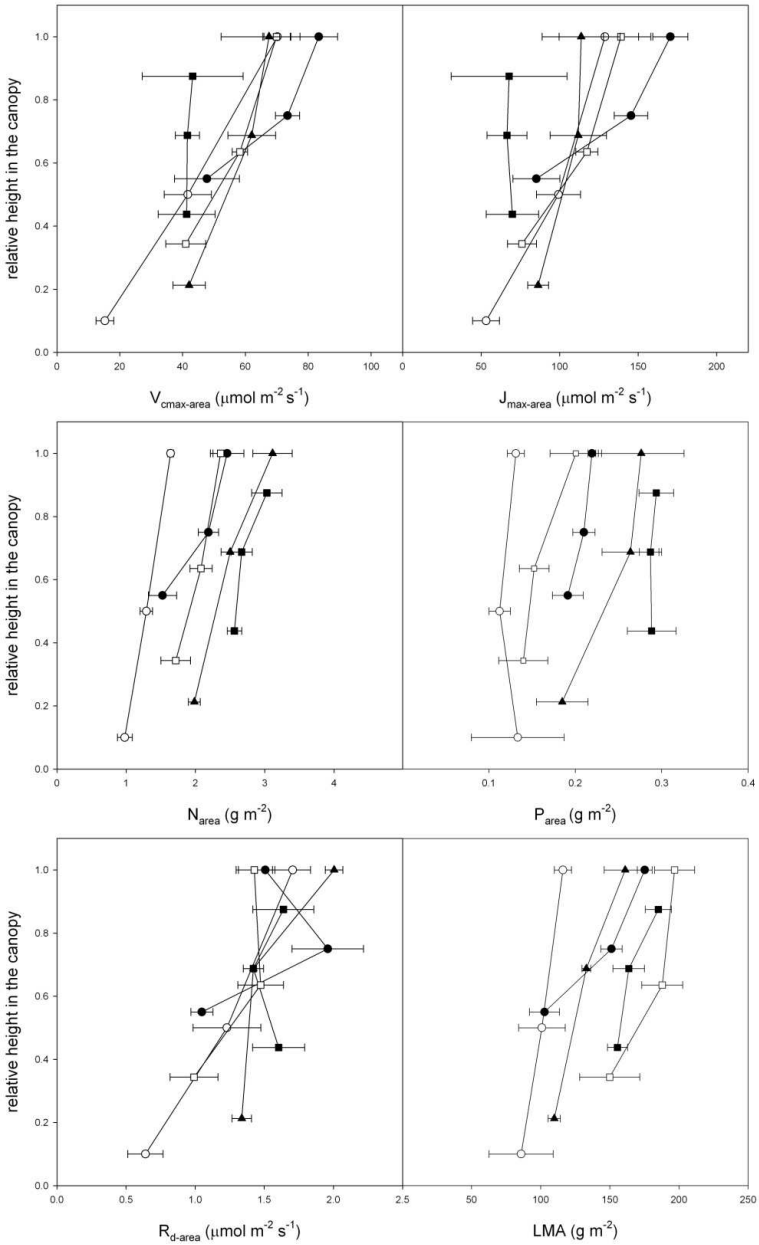

Figure 1. Photosynthetic parameters (V_{cmax} and J_{max}) and leaf dark respiration (R_d) standardised for 25 °C, leaf mass per area (*LMA*) and nitrogen and phosphorus content on a leaf area basis throughout the canopy per measured species ± SE, n = 5. Relative height = 1.0 indicates the top of the canopy. All parameters showed a significant correlation with canopy height ($P<0.05$). Black circles indicate the species *Schefflera allocotantha*, white circles indicate *Weinmannia bangii*, black squares indicate *Prunus integrifolia*, white squares indicate *Clethra cuneata*, and black triangles indicate *Clusia creatosa*.

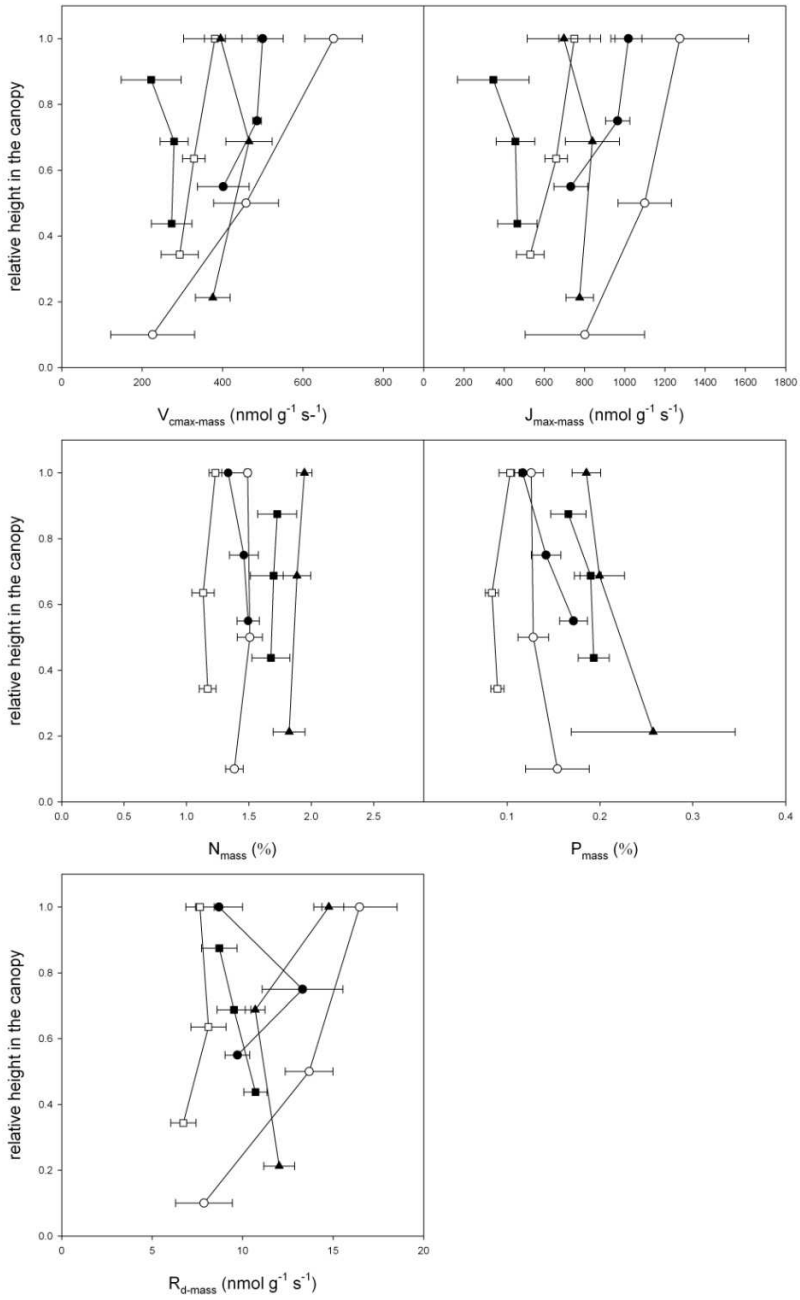

Figure 2. Photosynthetic parameters (V_{cmax} and J_{max}) and leaf dark respiration (R_d) standardised for 25 °C, and nitrogen and phosphorus content on a mass basis throughout the canopy per measured species ± SE, n = 5. Relative height = 1.0 indicates the top of the canopy. None of the parameters showed a significant correlation with canopy height. Symbols are as in Figure 1.

1.61-1.99. No significant difference in $V_{cmax\text{-}area}$-$J_{max\text{-}area}$ relationships was found between leaves from top, middle or bottom canopy level and although average $V_{cmax\text{-}area}$ and $J_{max\text{-}area}$ varied amongst species, no significant differences in the $V_{cmax\text{-}area}$-$J_{max\text{-}area}$ ratio could be detected between them. After normalising both $V_{cmax\text{-}area}$ and $J_{max\text{-}area}$, the extinction coefficients (k_p) for both parameters were 0.13 and 0.11, respectively, with no significant difference between both k_p values.

Table 1. Estimated intercepts and coefficients ±SE (n =78) for the three level random intercept and random coefficient model for changes in leaf traits (ln-transformed) with canopy height on an area and mass basis (Snijders and Bosker, 1999). Bold values indicate significant coefficients (P<0.05), and an asterisk indicates a significant improvement of the model by including the random factors 'trees' and 'species'. The model was centred around the average height of the canopy (10.9 m)

Parameter	Intercept (β0)	SE	Coefficient (β1)	SE
Area basis				
LMA	4.904	0.084	**0.073***	**0.016**
N_{area}	0.618	0.107	**0.091**	**0.013**
P_{area}	-1.736	0.141	**0.057**	**0.020**
$V_{cmax\text{-}area}$	3.484	0.295	**0.089***	**0.050**
$J_{max\text{-}area}$	4.107	0.222	**0.079***	**0.033**
$R_{d\text{-}area}$	-0.95	0.051	**0.097**	**0.025**
Mass basis				
N_{mass}	2.631	0.094	0.006	0.011
P_{mass}	0.281	0.121	-0.018	0.014
$V_{cmax\text{-}mass}$	-1.386	0.089	-0.005	0.038
$J_{max\text{-}mass}$	-0.722	0.259	-0.046	0.037
$R_{d\text{-}mass}$	-4.976	0.07	0.018	0.017

Compared with the photosynthetic parameters, R_d showed less variation amongst species (Figure 3 and 4), though interspecies differences were larger when R_d was expressed on a mass basis ($R_{d\text{-}mass}$) than on an area basis ($R_{d\text{-}area}$). Average $R_{d\text{-}area}$ was 1.44 ± SE 0.05 μmol m^{-2} s^{-1} and $R_{d\text{-}mass}$ 10.4 ± SE 0.42 nmol g^{-1} s^{-1} when corrected to 25 °C. For the ambient temperature of 12.5 °C, $R_{d\text{-}area}$ was estimated to be 0.57 ± SE 0.02 μmol m^{-2} s^{-1}, while $R_{d\text{-}mass}$ was 7.12 ± SE 0.28 nmol g^{-1} s^{-1}. The relationship of $R_{d\text{-}area}$ and canopy height was significant (P<0.05, table1), but there was no significant difference between a fixed or random β1, which implies that including

inter and intra-species variance did not improve the multilevel regression model, unlike the photosynthetic parameters $V_{cmax\text{-}area}$ and $J_{max\text{-}area}$.

3.3.2 Foliar N, P and leaf mass per area

Figure 1 and 2 show the results of the foliar N and P analyses, as well as the *LMA* results. N_{area}, $P_{area,}$ and *LMA* all showed similar significant positive relationships with canopy height ($P<0.05$), but for P_{mass} and N_{mass} this was not observed (Table 1). For *LMA*, the relationship was better explained when the regression coefficient β1 was allowed to vary amongst the species. This was not observed for the analysis of N_{area} or P_{area} (Table 1), even though a large part of the variance in N_{area} and P_{area} resulted from inter-species differences (Figure 3). For P_{area} (though to a lesser extent for P_{mass}) a relatively large portion of the variance was explained by intra-species differences, compared with the other measured variables (Figure 3 and 4).

3.3.3 Relationships among V_{cmax}, J_{max}, R_d, N, P and LMA

The strongest observed positive bivariate relationships were between the log-transformed $V_{cmax\text{-}area}$ and $J_{max\text{-}area}$ ($R^2 = 0.729$, n = 78; Table 2) and $V_{cmax\text{-}mass}$ and $J_{max\text{-}mass}$ ($R^2 = 0.726$, Table 3). $V_{cmax\text{-}area}$ was significantly related to N_{area} and *LMA*, although the fit of both these relationships was noisy ($R^2 = 0.10$ and $R^2 = 0.14$, respectively) and no relationship between $V_{cmax\text{-}area}$ and P_{area} was found (Table 2). When plotting the untransformed $V_{cmax\text{-}area}$ and N_{area} data (Figure 6), a relationship often used for predicting V_{cmax}, the relationship was significant but very patchy ($V_{cmax\text{-}area} = 31.02 + 10.8 * N_{area}$, $P<0.05$, $R^2 = 0.094$). When analysed by species, only the $V_{cmax\text{-}area}$-N_{area} relationship for *Prunus integrifolia* was not significant ($P=0.94$, n = 14), while for the other four species it was ($P<0.05$, n = 64). Removing *Prunus integrifolia* from the dataset increased the R^2 of the $V_{cmax\text{-}area}$-N_{area} relationship from $R^2 = 0.094$ to $R^2 = 0.229$. $V_{cmax\text{-}mass}$ showed no significant relationship with N_{mass}, P_{mass} or *LMA*. $J_{max\text{-}area}$ showed no significant bivariate relationship between any of the other measured variables except for $V_{cmax\text{-}area}$ and $R_{d\text{-}area}$, and while $J_{max\text{-}mass}$ and *LMA* showed a significant positive bivariate relationship, this fit was poor ($R^2 = 0.08$; Table 3). The bivariate relationship between $R_{d\text{-}area}$ and N_{area} was stronger than

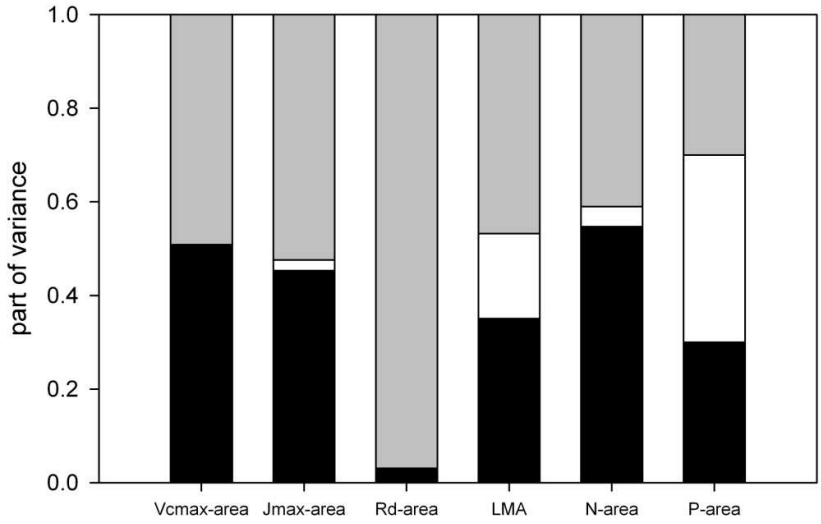

Figure 3. Partitioning of the variance from the total dataset on an area basis, according to a three level multilevel modelling approach (Snijders and Bosker, 1999). Grey parts indicate the variability with height within individual trees, white parts indicate variability between trees within the species, and black parts indicate variability between measured species.

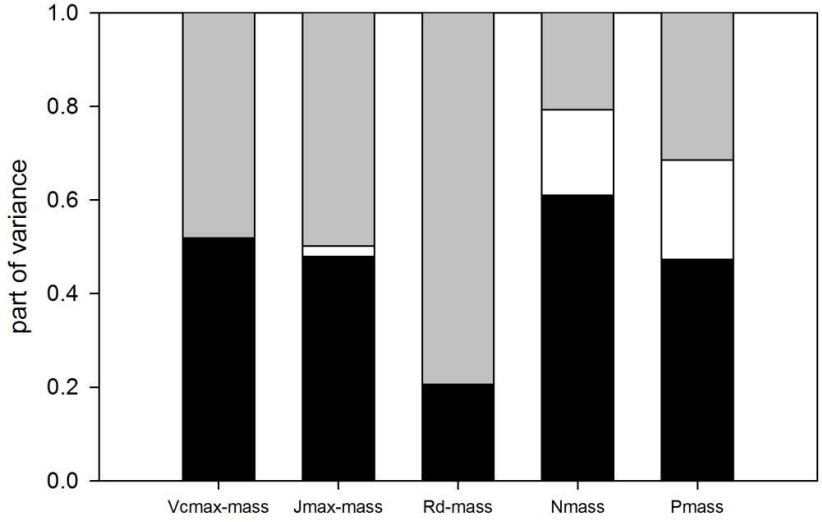

Figure 4. Partitioning of the variance from the total dataset on a mass basis, according to a three level multilevel modelling approach (Snijders and Bosker, 1999). Grey parts indicate the variability with height within individual trees, white parts indicate variability between trees within the species, and black parts indicate variability between measured species.

for $V_{cmax\text{-}area}$ and N_{area} (R^2 = 0.43). $R_{d\text{-}area}$ also showed a significant bivariate relationship on the log-transformed data with P_{area} and *LMA* (R^2 = 0.19 and R^2 = 0.26, respectively; Table 2). For $R_{d\text{-}mass}$ significant bivariate relationships were also present, but the strengths of the fits, as determined by the R^2 coefficients, were weaker (Table 3).

3.4 Discussion

3.4.1 Foliar CO_2 exchange parameters

When expressed on an area basis, the mean $V_{cmax\text{-}area}$ and $J_{max\text{-}area}$ values (55.1 ± SE 2.6 and 106.5 ± SE 5.3 μmol m^{-2} s^{-1}, respectively) were within the range reported for lowland rainforests (Table 4) when standardised to 25 ºC. This supports earlier assumptions that photosynthetic capacity in TMCFs is not systematically lower than in lowland rainforests (Bruijnzeel and Veneklaas 1998), although this result does not hold when expressing V_{cmax} and J_{max} on a mass basis, since the average TMCF *LMA* is relatively high (Table 4). This phenomenon has been observed in other studies comparing TMCF leaf traits, like N_{area} and N_{mass}, with those from lower altitudes (Tanner *et al.* 1998, Kitayama and Aiba 2002, van de Weg *et al.* 2009). Higher *LMA* values have been considered as a plant strategy for increasing leaf longevity, and thereby optimising the use of relatively scarce nutrients (*e.g.* Kikuzawa 1991, Reich *et al.* 1992, Reich 1993); expressed on a mass basis, foliar N has generally been shown to be lower in TMCFs (Tanner *et al.* 1998, Kitayama and Aiba 2002, van de Weg *et al.* 2009). The higher *LMA* values therefore enhance the photosynthetic biomass per unit leaf area to a value where it does not differ from lowland forests when expressed at a similar reference temperature. However, the ambient temperatures a TMCF experiences are lower than in tropical lowland forests. Air temperatures at the research site seldomly exceeds 20°C, and mean annual air temperature is 12.5 °C. Therefore, when both $V_{cmax\text{-}area}$ and $J_{max\text{-}area}$ are standardised to 12.5 °C, the photosynthetic parameters in TMCFs are in fact much lower are lower than in tropical lowland forests at thir ambient temperatures (Table 4).

Table 2. The bivariate relationships between log-transformed leaf traits on an area basis from the total dataset. Standardized major axis (SMA) slopes and their 95% confidence intervals are given in the upper right section of the matrix (*x*–axis variables are given in the rows; *y*-axis variables are in the columns. The inverse of these slopes describe the regression coefficient from *y* on *x*). The adjusted R^2 values for significant bivariate relationships ($P<0.05$) are given in the lower left section of the matrix (n =78). Non-significant relationships are indicated with the abbreviation NS.

Log/Log	V_{cmax}	J_{max}	R_d	*N*	*P*	*LMA*
V_{cmax}	-	0.96 (1.07, 0.85)	1.75 (2.12, 1.38)	1.88 (2.28, 1.48)	NS	1.90 (2.28, 1.51)
J_{max}	0.729	-	NS	NS	NS	NS
Rd	0.141	0.032	-	1.07 (1.26, 0.89)	0.76 (0.91, 0.60)	1.08 (1.30, 0.87)
N	0.096	NS	0.432	-	0.70 (0.82, 0.59)	1.01 (1.15, 0.87)
P	NS	NS	0.199	0.475	-	1.43 (1.71, 1.16)
LMA	0.162	NS	0.259	0.606	0.275	-

Table 3. The bivariate relationships between log-transformed measured leaf traits on a mass basis from the total dataset. See Table 2 for explanation of the table layout.

Log/Log	V_{cmax}	J_{max}	R_d	*N*	*P*	*LMA*
V_{cmax}	-	0.86 (0.96, 0.76)	NS	NS	NS	NS
J_{max}	0.726	-	NS	NS	NS	2.03 (2.46, 1.59)
Rd	NS	NS	-	1.53 (1.82, 1.23)	0.83 (1.02, 0.65)	1.03 (1.24, 0.82)
N	NS	NS	0.297	-	0.55 (0.65, 0.45)	0.68 (0.82, 0.53)
P	NS	NS	0.092	0.0318	-	NS
LMA	NS	7.6	0.174	0.091	NS	-

A similar observation holds true for foliar dark respiration. Standardised to 25 °C, $R_{d\text{-}area}$ is higher than observed in lowland forests in Brazil and Costa Rica (Table 4), though of a similar value when standardised for the mean ambient temperature of 12.5 °C, indicating that $R_{d\text{-}area}$ values are of a similar magnitude in both montane and lowland tropical forests. However, the relatively lower ambient $V_{cmax\text{-}area}$ and $J_{max\text{-}area}$ and similar $R_{d\text{-}area}$ values in the TMCF, do not necessarily imply lower carbon use efficiencies for TMCF leaves compared with other tropical forests. Firstly, the net C-uptake (*A*) will depend not only on the TMCF photosynthetic capacity, but environmental conditions (*i.e.* PAR and relative humidity) will influence the *in situ* leaf level C-uptake. Moreover, *A* is the sum of gross photosynthesis, photorespiration and light respiration (R_l). R_l is lower than R_d, but has a lower Q_{10} than R_d (Atkin *et al.* 2002, Atkin *et al.* 2006). How this translates to daytime or 24 hour carbon use efficiency remains unclear given the lack of data on TMCF R_l.

The slope of the observed TMCF $J_{max\text{-}area}$-$V_{cmax\text{-}area}$ relationship was slightly steeper (1.80 ± 0.1, type I regression,) but not significantly different from the global relationship found by Wullschleger *et al.* (1993) or Meir *et al.* (2002) (Figure 5). However, when comparing the TMCF $J_{max\text{-}area}$ -$V_{cmax\text{-}area}$ more specifically to other tropical studies, the observed slope is significantly steeper ($P<0.05$) than found for a wide range of sun-lit leaves from savannah species (Domingues *et al.* 2010) and leaves from the whole canopy in a mature lowland rain forest species (Domingues *et al.* 2007), but similar to lowland rainforest saplings, which are accustomed to grow in the shade (Coste *et al.* 2005). This suggests that TMCF foliage may be more similar to understorey plants with respect to additional investment into electron transport capacity (J_{max}), than Rubisco activity (V_{cmax}), when compared to other mature tropical forest trees. TMCFs have been hypothesized to be light limited, since average daily received photosynthetic active radiation (PAR) values are relatively low (Bruijnzeel and Veneklaas 1998, Letts and Mulligan 2005) and the higher investments in J_{max} compared to V_{cmax} could be an adaptation to these lower PAR levels. However, consistent with other studies (Evans and Poorter 2001, Niinemets *et al.* 2004), no shift in J_{max}-V_{cmax}-ratios were observed at canopy level, showing that there was no *in situ* acclimation to the lower light levels at the bottom of the canopy.

It is therefore unlikely that the relatively high TMCF J_{max}-V_{cmax} slope for the whole canopy is an acclimation to lower light levels, though they could represent a genetic adaptation from TMCF species.

Table 4. Average $V_{cmax\text{-}area}$, $J_{max\text{-}area}$, $R_{d\text{-}area}$ standardised for 25 °C (μmol m^{-2} s^{-1}) and *LMA* (g m^{-2}) values from tropical lowland forests found in the literature, together with the average values of this study standardised for T_{leaf} = 25 °C and 12.5°C, with their standard deviations. The $V_{cmax\text{-}area}$ and $J_{max\text{-}area}$ values from Domingues *et al.* (2007) and Meir *et al.* (2007) were adjusted to 25 °C according to Bernacchi *et al.* (2003). The average *LMA* for Cavaleri *et al.* (2008) was recalculated from the regression function between $R_{d\text{-}area}$ and *LMA* given in that study.

Publication	**Ecosystem and location**	$V_{cmax\text{-}area}$ **(μmol m^{-2} s^{-1})**	$J_{max\text{-}area}$ **(μmol m^{-2} s^{-1})**	$R_{d\text{-}area}$ **(μmol m^{-2} s^{-1})**	***LMA*** **(g m^{-2})**
This study T_{leaf} =25 °C	Tropical montane cloud forest, Peru	55.6 ± 23.03	106.5 ± 47.2	1.47 ± 0.52	145.03 ± 37.2
This study T_{leaf} =12.5 °C	Tropical montane cloud forest, Peru	17.45 ± 7.21	50.51 ± 21.74	0.57 ± 0.20	
Coste *et al.* (2005)	Rainforest, French Guiana (saplings)	22.1-55.3	110 ± 40		44.6-117
Domingues *et al.* (2007)	Rainforest, Brazil (upper canopy only)	52.46 ± 18.5	83.63 ±32		129.5 ± 21.7
Carswell *et al.* (2000)	Rainforest, Brazil	42.8 ± 5.9	97.2 ± 12		114-69
Meir *et al.* (2007)	Rainforest, Cameroon	49-68	78-133		49-76
Cavaleri *et al.* (2008)	Rainforest, Costa Rica			0.41 ± 0.25	~ 57.5
Meir *et al.* (2001)	Rainforest, Brazil and Cameroon			0.11-0.78 0.22-1.19	90-110

An alternative hypothesis is that the TMCF leaves do not need to invest as much in V_{cmax} compared to the savannah or tropical rain forest leaves, because stomatal conductance remains relatively high through the day (data not shown). As a consequence, TMCF leaves experience higher C_i values and need less investment in Rubisco to acquire sufficient levels of carboxylation rates. However, with experimental data lacking, both explanations remain speculative.

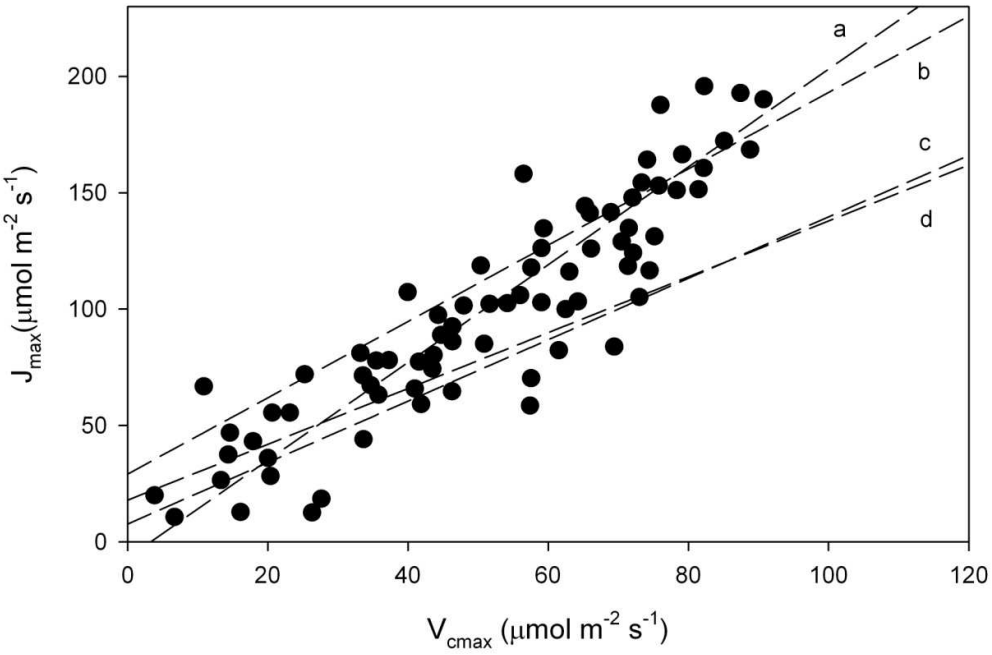

Figure 5. $V_{cmax\text{-}area}$ and $J_{max\text{-}area}$ values from this study, together with $V_{cmax\text{-}area}$-$J_{max\text{-}area}$ relationships from (a) tropical rainforests saplings (Coste *et al.* 2005), (b) a global dataset (Wullschleger 1993), (c) tropical rainforest species from Santarem, Brazil (Domingues *et al.* 2007), savannah species West-Africa (Domingues *et al.* 2010).

3.4.2. Canopy Profile

Consistent with studies from other ecosystems (*e.g.* Meir *et al.* 2002, Domingues *et al.* 2005, Han *et al.* 2006), all measured variables had a significant relationship with canopy height when expressed on an area basis, but not on a mass basis. The driver for this difference is the change in *LMA* throughout the TMCF canopy (Figure 1). High *LMA* values under high PAR (*i.e.* top of canopy) have shown to increase

photosynthesis through concentrating the light harvesting capacity per area, while growing under low PAR conditions, low *LMA* values increase the light harvesting efficiency of a leaf much more than higher N investments in light harvesting complexes would do (Evans and Poorter 2001, Niinemets 2007). Since the leaf tissue density did not change throughout the canopy in our study site (data not shown), the light gradient within a canopy is probably most important for determining the observed pattern of *LMA*. Less of the variance in the dataset of $R_{d\text{-}mass}$ and $R_{d\text{-}area}$ could be attributed to inter-species variance, compared with the photosynthetic parameters. This is consistent with studies on fast and slow growing species that also showed a larger part of the variance in photosynthesis explained by species differences than for foliar respiration (Poorter *et al.* 1995). It indicates that species specific knowledge is more important for estimating TMCF V_{cmax} and J_{max} than for estimating R_d. However, how the inter-species variance would be expressed in R_l remains unclear from these results, so for the total foliar respiration this might be equally important.

No data are available for the light levels within the TMCF canopy, which prevented a test of how well the photosynthetic parameters fit with the light distribution in the canopy. However, the observed extinction coefficient (k_p) for both $V_{cmax\text{-}area}$ and $J_{max\text{-}area}$ were relatively low (0.13 and 0.11, respectively), indicating that like many other studies (Dejong and Doyle 1985, Hollinger 1996, Meir *et al.* 2002, Anten 2005), their relationships with height are not as tight as the optimisation theory of Field (1983) predicts. However, our results agree with the model that was put forward recently by Lloyd *et al.* (2009). According to this model, total canopy C-uptake can either increase or decrease with a given A_{sat} depending on the combination of the k_p and LAI. Since the TMCF has similar standardised $V_{cmax\text{-}area}$ and $J_{max\text{-}area}$ values to tropical lowland rainforests, but a lower LAI, a lower TMCF k_p would give a higher TMCF C-uptake than the k_p=0.15 that Lloyd *et al.* (2009) used for lowland rainforests.

3.4.3 Relationships between nutrients and foliar gas exchange

The relationship between photosynthetic parameters and N and the lack of relationship with P are consistent with the general hypothesis of Tanner *et al.* (1998), which stated that TMCFs are likely to be N- rather than P-limited. Our data suggest that the trees in the study area have enough P available to maintain their photosynthesis despite the likely reduced rate of phosphate recycling resulting from the relatively low ambient temperatures (Savitch *et al.* 1997, Strand *et al.* 1997, Sage and Kubien 2007). TMCF soils probably have more P available because they are not as old and leached as observed in tropical lowland forests (Sanchez 1979, Wardle *et al.* 2004). As a consequence, a much weaker relationship between photosynthetic capacity and leaf P might be expected, as found in selected tropical lowland forests where P availability is also thought to be high (*e.g.* Meir *et al.* 2007). Because of the lack of relationship between V_{cmax} and P, we did not attempt to analyse our dataset in the same fashion as Domingues *et al.* (2010), who presented a new model to test for independent constraints between P and N on V_{cmax}. The significant $V_{cmax\text{-}area}$ -N_{area} relationship suggests that the amount of foliar N is controlling TMCF C-uptake, although the fit of $V_{cmax\text{-}area}$ with N_{area} was not tight when *Prunus integrifolia* was included in the dataset (Figure 6). Although we do not have data on the N partitioning of the studied TMCF species, species from high altitudes and with high *LMA* are known to invest less N in their photosynthetic apparatus, but more in cell wall components and (Westbeek *et al.* 1999, Takashima *et al.* 2004). However, Harrison *et al.* (2009) found little evidence that allocation of N to cell walls is responsible for variations in nitrogen use efficiency (NUE) and even if a lower overall investment in Rubisco by plants from higher altitudes can explain a less steep fit of $V_{cmax\text{-}area}$ and N_{area}, it does not account for the noisy fit of the total dataset. There are other nitrogenous compounds in leaves(proteins, secondary compounds), and species from the *Rosaceae* family frequently invest N in cyanogenic glycosides, which is a protective nitrogenous compound (Vetter 2000). Cyanogenic glycosides have been observed in other tropical *Prunus* species (Fransisco 2000, Miller 2004), and the investment is this compound is known to differ with leaf age. Therefore, although we took great care in only analysing full grown *Prunus integrifolia* leaves, it is possible that because of irregular investments in protective nitrogenous

compounds like cyanogenic glycosides there was no $V_{cmax\text{-}area}$-N_{area} relationship found for this species.

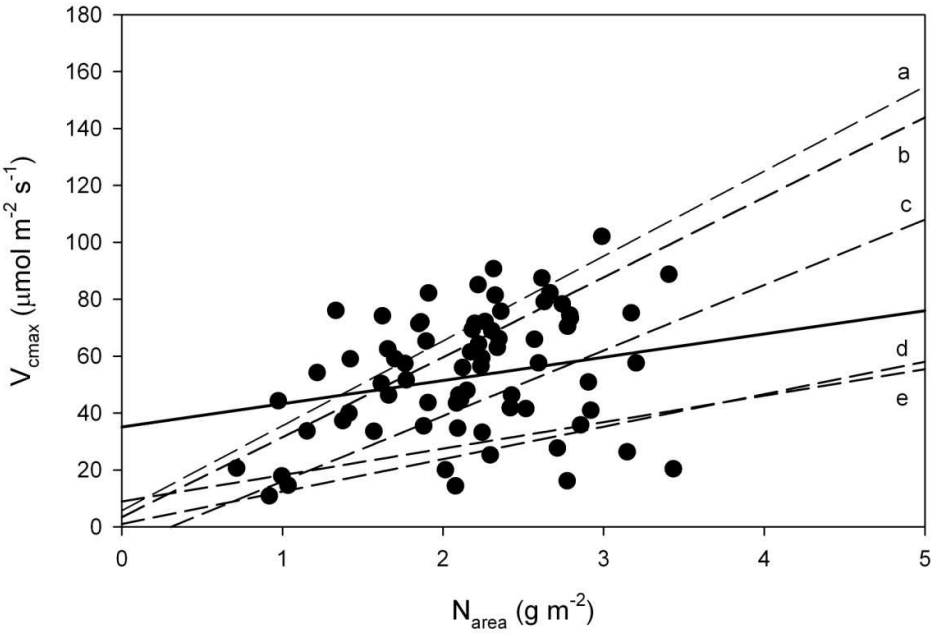

Figure 6. $V_{cmax\text{-}area}$ (µmol m-^{2} s^{-1}) standardised for the reference temperature of 25 °C and N_{area} (g m^{-2}) values from this study. The solid line represents the regression function $V_{cmax\text{-}area}$ = 31.02 + 10.8* N_{area} ($P<0.05$, $R^2 = 0.094$). The dashed lines represent $V_{cmax\text{-}area}$-N_{area} relationships from (a) temperate broadleaf species (Kattge *et al.* 2009), (b) nonoxisol tropical rainforest in Borneo, Malaysia (Kumagai *et al.* 2006) , (c) oxisol tropical rainforest in Tapajos, Brazil, (Domingues *et al.* 2005) , (d) oxisol tropical rainforest in Manaus, Brazil (Carswell, 2000) and (e) oxisol tropical rainforests (Kattge *et al.* 2009).

The $V_{cmax\text{-}area}$-N_{area} relationship from our study does not resemble those observed in either tropical or temperate ecosystems (Figure 6 and 7). On a global scale, both leaf structural parameters and climatic factors have shown to explain the variance in photosynthetic leaf traits (Wright *et al.* 2004, Wright *et al.* 2005, Reich *et al.* 2007). TMCFs have relatively high *LMA* values, and consequently similar N_{area} values compared with tropical lowland forests (van de Weg *et al.* 2009). Therefore, we would have expected the $V_{cmax\text{-}area}$ at 25 °C to be lower in TMCFs than in lowland tropical forests, because of the higher investment of N in cell wall and other

compounds by leaves with higher *LMA*. This holds true when comparing the TMCF $V_{cmax\text{-}area}$-N_{area} relationship with tropical lowland rain forests growing on nonoxisols (Kumagai *et al.* 2006, Kattge *et al.* 2009), but not for those growing on nutrient-poor oxisols (Carswell *et al.* 2000, Domingues *et al.* 2005), which actually predict lower $V_{cmax\text{-}area}$ values at a similar N_{area} (Figure 6). More important in explaining the different TMCF $V_{cmax\text{-}area}$ –N_{area} relationship is the lower mean annual temperatures TMCFs experience. When standardised to 12.5 °C, the TMCF photosynthetic parameters were reduced to values much lower (Table 4) than observed in tropical lowland forests, although their N_{area} stays the same. On a global scale, leaves from colder biomes have higher photosynthetic nitrogen use efficiencies (NUE) in order compensate for reduced Rubisco activity because of lower temperatures and hence to optimise their C-uptake (Kerkhoff *et al.* 2005). Therefore, it would be expected that the $V_{cmax\text{-}area}$-N_{area} ratio from a TMCF fits between tropical and temperate ecosystems. Indeed, the observed $V_{cmax\text{-}area}$-N_{area} ratio (24.8 ± SE 1.18) and average observed $V_{cmax\text{-}area}$ fit within the plant functional type (PFT) classifications made by Kattge *et al.* (2009), and within the spectrum of their study could be qualified as a PFT of its own (Figure 7). Overall, the higher NUE value at the reference temperature of 25 °C implies that TMCF leaves reserve more N per unit N for photosynthesis than tropical lowland forests do as an adaptation in order to optimize their C-uptake at the lower temperatures they experience. Furthermore, it emphasises that neither NUE values from tropical lowland forests nor temperate forests predict TMCF V_{cmax} values adequately.

Estimating V_{cmax} accurately is important when modelling GPP (Kattge *et al.* 2009, Mercado et al, 2009), and even though TMCFs only cover up to 11% of tropical forests worldwide (Doumenge *et al.* 1995), the interest in the C-cycle of TMCFs with regard to carbon sequestration and storage practices has increased substantially in recent years (*e.g.* Fehse *et al.* 2002, Martínez *et al.* 2009). The results reported here can increase the accuracy of estimating TMCF C-uptake, especially for Andean TMCFs, where other studies have shown similar N:P ratios that indicate N limitation (*i.e.* N:P = 8-10, Soethe *et al.* 2008). For TMCFs with relatively high N:P ratios (i.e.

29-96, Kitayama and Aiba 2002, Proctor *et al.* 1989), however, V_{cmax} might be estimated better with a model that includes foliar P.

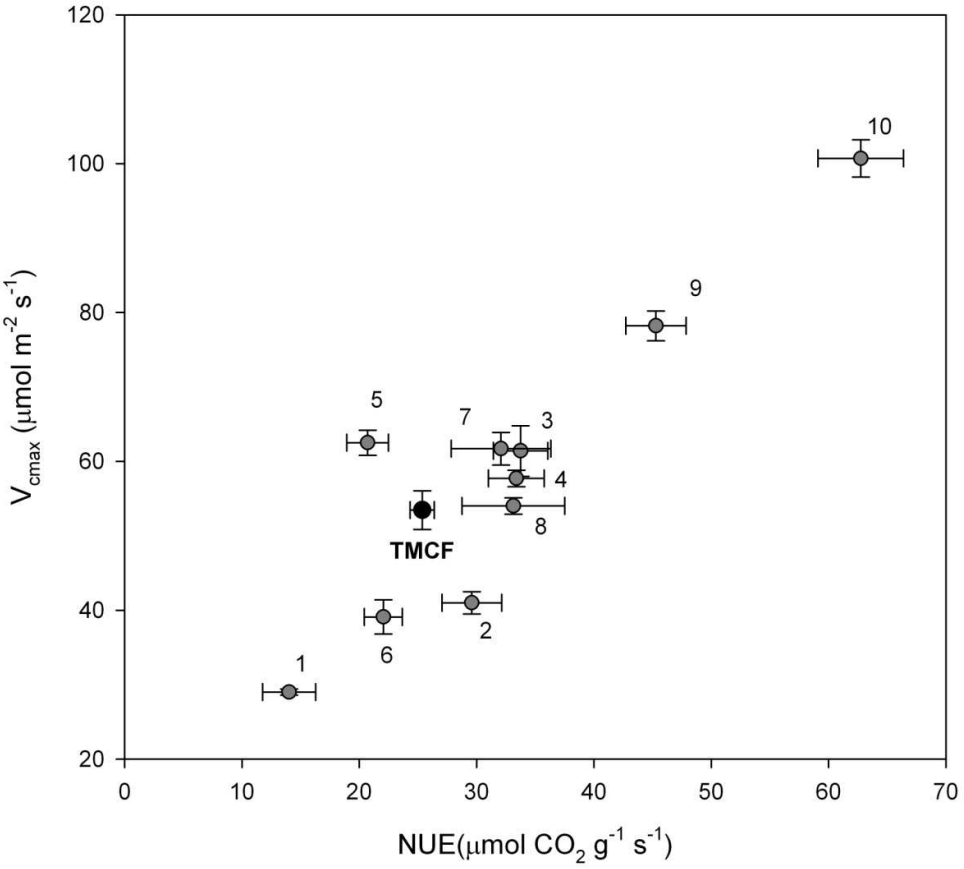

Figure 7. $V_{cmax\ area}$ standardised for 25 °C (µmol m-^{2} s^{-1}) against nitrogen use efficiency (NUE, i.e. $V_{cmax\ area}/N_{area}$) from this study (TMCF) ± SE (n = 78), together with data from global plant functional types with standard error bars error bars from Kattge *et al.* (2009). Plant function types are: (1) tropical trees (oxisols), (2) tropical trees (nonoxisols), (3) temperate broadleaved evergreen trees, (4) temperate broadleaved deciduous trees, (5) evergreen coniferous trees, (6) deciduous coniferous trees, (7) evergreen shrubs, (8) deciduous shrubs, (9) C_3 herbaceous, (10) C_3 crops.

3.5 Conclusions

This study presents a first published dataset for V_{cmax} and J_{max} values of Andean TMCFs. The photosynthetic parameters do not differ from published values from

tropical lowland rainforests when expressed on an area basis and standardised for the same reference temperature. At ambient temperatures, however, TMCF V_{cmax} and J_{max} are lower compared with the ambient values from tropical lowland forests, as a consequence of lower Rubisco activity at lower temperatures. The lack of difference at standardised temperatures is firstly explained by the relatively high *LMA* values found in the TMCF, which concentrates the N per leaf area. Additionally, the TMCF NUE is larger than in tropical lowland forests, indicating an adaptation to the lower mean annual TMCF temperature and. Unlike other tropical forests, the TMCF leaves appear to invest more of their resources in the electron transport part of the photosynthetic apparatus than the carboxylation component, since the $V_{cmax\text{-}area}$-$J_{max\text{-}area}$ relationship was significantly steeper. As observed in many other forest ecosystems, all measured leaf traits showed a significant relationship with canopy height when expressed on an area basis, but not on a mass basis. For the photosynthetic parameters $V_{cmax\text{-}area}$ and $J_{max\text{-}area}$, this relationship with canopy height varied between species, but the same variation was not observed for $R_{d\text{-}area}$, although this does not necessarily translate to less inter-species variance in the total foliar C-output, since data on R_l was unavailable. However, it underlines the importance of a sampling from a large enough species pool when quantifying the photosynthetic capacity of a TMCF, in order to capture all variability. Finally, a significant, though noisy, relationship between $V_{cmax\text{-}area}$ and $N_{\text{-}area}$ was found, which is consistent with the hypothesis of Tanner *et al.* (1998) where TMCF productivity, unlike that for tropical lowland forests, is thought to be limited by N. The V_{cmax}-N relationship also showed that when predicting TMCF C-uptake, V_{cmax}-N relationships from both temperate and tropical lowland forests do not predict TMCF V_{cmax} adequately.

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Chapter 4

Paper 3: Stomatal conductance, photosynthesis and leaf water potential in four tropical montane cloud forest species in Peru

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Abstract

The frequent occurrences of cloud events make tropical montane cloud forests (TMCF) a hydrologically unique ecosystem. Although the hydrological functioning of TMCFs has been the subject of research for decades, there are still questions on how the TMCF environment influences diurnal leaf water potential (Ψ_{leaf}), stomatal conductance (g_s) and consequently, net foliar photosynthesis (A). In this study, environmental variables (air temperature (T_{air}), vapour pressure deficit (VPD), photosynthetic photon flux density (PAR), soil water content (SWC)) and plant responses (Ψ_{leaf}, g_s, and A) for four TMCF species were measured throughout the day at 2990 m a.s.l. in a TMCF in south east Peru during the dry season. The diurnal patterns of Ψ_{leaf} differed amongst the TMCF species, with *Clusia cretosa* and *Schefflera allocotantha* having isohydric diurnal patterns of Ψ_{leaf} and g_s, while *Weinmannia crassifolia* and *Clethra cuneata* showed anisohydric behaviour. For all four species, g_s was not very sensitive to changes in VPD or SWC, and no pattern of midday depression was observed for either g_s or A. This implies that in this study site g_s, and consequently A and thus carbon gain, is not controlled by the atmospheric demand or drought stress under the current dry season conditions.

Keywords: stomatal conductance, tropical mountain cloud forests, photosynthesis, leaf water potential, Peru, *Clusia cretosa*, *Schefflera allocotantha*, *Weinmannia crassifolia*, *Clethra cuneata*

4.1 Introduction

Tropical montane cloud forests (TMCF) are unique in their hydrological functioning. Their frequent cloud cover causes a high relative humidity (RH), reduced levels of radiation and hydrological input through a phenomenon called ‘cloud stripping’, which occurs when water vapour condensates on the vegetation (*e.g.* Stadtmüller 1987, Hamilton *et al.* 1995, Bruijnzeel 2001, Foster 2001). Both structural factors (*e.g.* low leaf area index (LAI)) and many of the environmental factors (*e.g.* low vapour pressure deficit (VPD) and low photosynthetically active radiation (PAR))

that are typical of TMCFs, have shown to correlate with relatively low transpiration rates in TMCFs in Hawaii, Puerto Rico, Indonesia and Ecuador (Bruijnzeel and Proctor 1995, Santiago *et al.* 2000, Schellekens *et al.* 2000, Motzer *et al.* 2005). Few data is available, however, on the stomatal conductance (g_s) in TMCFs, even though stomatal opening and closure is the biological control on transpiration.

Stomata are important for both a plant's hydraulic functioning and its carbon (C) uptake, because they regulate the diffusive conductance from the environment to the intercellular space and *vice versa*, and thereby influence both the water loss from a leaf (transpiration) and its C-uptake. Although the exact way through which stomatal closure is regulated by the involved signalling cascades is still up for discussion (Buckley 2005, Lawson 2009), it is well known that the stomatal aperture is sensitive to a set of (micro)meteorological and physiological factors. Stomata respond to changes in VPD in order to prevent excessive water loss (*e.g.* Cowan 1977, Monteith 1995, Jones 1998, Shope *et al.* 2008), while light, independently from other factors, induces stomatal opening to promote photosynthesis (Wong *et al.* 1979, Zeiger 1983, Shimazaki *et al.* 2007). The relationship between light and g_s is both direct, with blue light and PAR causing stomatal opening through light transduction pathways (Zeiger *et al.* 2002, Shimazaki *et al.* 2007), and indirect through a CO_2 'feedback loop'. In this feedback loop, the reduction in intercellular CO_2 as a consequence of photosynthesis causes the stomata to open (Baroli *et al.* 2008). In addition, it has been well established that both leaf water status and soil water potential are essential regulating factors of stomatal closure, especially under drought stress. Absiscic acid (ABA) that is produced in the root tips as a response to drought is transported through the xylem to the leaves where it initiates a various set of signalling proteins that initiate stomatal closure (*e.g.* Davies et al, 1990, Davies &Zhang, 1991, Comstock 2002, Li *et al.* 2006). However, the relative importance of all environmental factors that influence g_s appears to be context dependent. Studies have shown that stomata can be induced to re-open after manipulation of the leaf water status, and stomatal sensitivity to modifications in intercellular CO_2 changes with soil water stress and different VPD values (Jarvis 1976, Saliendra *et al.* 1995, Fuchs and Livingston 1996, Mencuccini *et al.* 2000, Hladnik et al, 2008). Additionally,

time of day and species specific responsivity are all known to influence stomatal opening and closure as well (Mott 1988, Osborne 1989, Correia *et al.* 1997, Mencuccini *et al.* 2000). For example, so called 'isohydric' species are known to control the stomatal aperture to a degree that regardless of the soil water status, the leaf water potential (Ψ_{leaf}) stays relatively high, while in 'anisohydric' species both leaf water potential and g_s decline with increasing drought stress (*e.g.* Tardieu and Simonneau 1998, Franks *et al.* 2007). In general, mesic environments are thought to host mostly isohydric species (Schulze *et al.* 1994). When analyzing the global distribution of the sensitivity of g_s to measured foliar ABA levels, Heilmeier *et al.* (2007) showed that tropical rainforest stomata are not very sensitive to changes in ABA and thus their g_s not very responsive to changes in soil water status. Phillips *et al.* (2001) found indeed that in two Panamanian species, g_s was not responsive enough to changes in soil water status in order to prevent cavitations of the xylem. On the other hand, a 1 ha scale drought experiment in Amazonia showed that rainforest species have an isohydric responses to drought (Fisher *et al.* 2006). In short, the importance of minimizing transpiration rates relative to photosynthetic carbon gain, together with the environmental factors controlling this, have been well recognized for decades. However, the relative importance of each controlling factor seems context dependent and can differ between species and biomes (Wong *et al.* 1979, Jarvis and Davies 1998, Sperry 2000, Buckley 2005, Hlandik *et al.* 2009).

For TMCF species, it is unknown whether they can be categorized as isohydric or anisohydric species. The few available studies on TMCF g_s have shown that g_s is rather insensitive to changes in VPD (Kapos and Tanner 1985, Motzer *et al.* 2005), or that g_s decreases with increases in VPD, but only to an extent where transpiration rates are not affected (Körner *et al.* 1983). Cavelier *et al.* (1990) studied the diurnal pattern of g_s and Ψ_{leaf} in a Colombian elfin forest. They indicated that in this forest type, leaf water status plays a role in controlling g_s. However, it is unclear whether this relationship also applies to cloud forests that occur below the elfin forest ecotone. Furthermore, although drought stress has been reported to cause tree death in upper montane rainforests, (Lowry *et al.* 1973, Werner 1988), periodic water deficiency is not thought to be a major control in TMCF functioning (Bruijnzeel and

Veneklaas 1998). In addition, not many studies have investigated TMCF *in situ* net photosynthesis (*A*), and how the diurnal pattern of g_s controls TMCF C-uptake. From the studies available, Letts and Mulligan (2005) showed that *A* varied from 3.5-10.6 μmol m^{-2} s^{-1} in north west Andean TMCFs, which is not unlike values reported from lowland rainforests, while in Borneo *A* at saturating light varied between 5.4-3.1 μmol m^{-2} s^{-1} (Hikosaka *et al.* 2002). Nonetheless, the interaction between leaf water status, g_s and *A* in TMCFs and how they vary amongst TMCF species remains little investigated and studies on the diurnal patterns of TMCF g_s, Ψ_{leaf} and *A* are lacking.

In this study, the range of *in situ* g_s, Ψ_{leaf} and *A* throughout the day in the dry season in a TMCF in south east Peru were examined in order to investigate whether leaf stomatal activity limits TMCF C-uptake. The research aims were: 1) To investigate for four abundant TMCF species whether they have similar or distinct patterns in their diurnal g_s and Ψ_{leaf} . 2) To test how TMCF g_s responds to the experienced ranges in Ψ_{leaf} and the environmental variables VPD, temperature, soil water content (SWC) and PAR. 3) To investigate whether TMCF *A* is limited by stomatal closure throughout the day in the dry season.

4.2 Methods

4.2.1 Fieldwork site

The study was performed in a tropical montane cloud forest located in the Kosñipata valley in south east Peru at 2990 m a.s.l. (13º11'28''S / 71º35'24''W), near the Wayquecha research station. The research site experiences a mean daily temperature of 12.5 ± SD 1.05 ºC, mean daily VPD is 0.14 ± SD 0.1 kPa and mean annual rainfall is 1706 mm yr^{-1}. A 'wet season' can be distinguished from October to March, with 46% of the rain falling from December to February. Soil water content (SWC) at 30 cm depth ranges from 0.21 to 0.41 with an annual average of 0.31, and at 1 m depth it ranges from 0.114 to 0.185 with an annual average of 0.139. A 1 ha plot has been previously established here, and the forest site is dominated by *Weinmannia* and *Clusia* species (Feeley and Silman, in press). Measurements for this study were

conducted on smaller individuals located next to this research plot in order to have access to intact leaves.

4.2.2 Environmental variables

Meteorological data was measured every 10 seconds and stored as an average every 10 minutes using an automated weather station (Campbell Scientific Ltd, U.K.) that was located within 50 m of all measured trees. Rainfall was measured with a tipping bucket rainfall gauge with a 0.2 mm resolution (Campbell Scientific Ltd, U.K.). Photosynthetically active radiation (PAR) was measured with a PAR quantum sensor (Skye Instruments Ltd, Powys, UK). Air temperature (T_{air}) and relative humidity (RH) were measured with a combined HMT sensor (Vaisala, Oy, Finland). Soil water content (SWC) was measured at 30 cm and 1 m depth with a water content reflectometer (CS616, Campbell Scientific Ltd, U.K.) and vapour pressure deficit (VPD) was calculated from that data by the data logger of the weather station (CR3000, Campbell Scientific Ltd, U.K.).

4.2.3 Leaf water potential, stomatal conductance and photosynthesis

The species selected for this study were *Weinmannia crassifolia*, *Clethra cuneata*, *Schefflera allocotantha*, and *Clusia creatosa*. Together, they represent approximately 48% of the local tree cover in the research plot, with *Weinmannia crassifolia* being the most dominant species (35%) and all selected trees were located within a radius of approximately 50 m of each other. For each species, g_s and Ψ_{leaf} were measured on 5 different individuals around 6:00 AM (predawn), 9:00 AM and 12:00 AM at two different leaves per tree in order to capture changes throughout the day. Because of time limitations, not all species could be measured the same day, but were spread between 2, 3 and 5 August 2008. A portable dynamic diffusion porometer (Delta-T AP4, Delta-T Devices Ltd, Cambridge, UK) was used to measure g_s. The porometer is equipped with a quantum PAR sensor, which enables an *in situ* PAR measurement for each g_s measurement and a thermocouple to measure leaf temperature (T_{leaf}). Only fully developed, non-damaged leaves were selected, and g_s was measured on

the abaxial sides of leaves after it had been determined that the g_s of the upper leaf surface was negligible. All g_s measurements were conducted on the central part of the blade, avoiding the midrib and margins of the leaf. After this measurement, the leaf was removed, put in a plastic zip-lock bag in order to avoid water loss, and directly measured for Ψ_{leaf} with a digital plant moisture system, also known as a 'pressure bomb' (Skye Instruments Ltd, Powys, UK). Because this is a destructive way of sampling, no repetitive measurements were possible for leaves included in this dataset. Additionally, on 8 and 10 August 2008, we measured Ψ_{leaf} and g_s on all four species, with only one selected tree per species at regular intervals (± 2 hours) between 6:00 and 16:00 on three leaves per tree. For safety reasons, no measurements were conducted in the field after 17:00.

Repetitive measurements for g_s were performed at five different individuals per species and at six leaves per tree (three 'sun' and three 'shade' leaves) at ± two hour intervals between 6:00 and 17:00 on 19, 21, 23 and 28 July and 14, 24 and 28 August 2008. Overall, for each species 240-300 measurements were conducted. In order to understand the variability of g_s between different leaves of the same species, we additionally performed 50 measurements around midday on one tree for each species. In addition, for five leaves per species, g_s measurements were conducted at five positions on the leaf in order to estimate within-leaf variance for each species.

For all four species *A* was measured in a similar fashion on 14, 24 and 28 August 2008, but only on three individual trees per species and on one or two sun and shaded leaf per tree due to the more laborious nature of these measurements (n = 30-45 per species). A portable photosynthesis machine (Li-Cor 6400, Li-Cor Inc, Lincoln, USA), fitted with a LED light source (6400-02B Red/Blue Light Source, Li-Cor, Inc, Lincoln, USA), was used to carry out the *in situ* photosynthesis measurements. The light source was set in the mode to follow the ambient PAR observed by the external quantum sensor of the Li-Cor 6400 head. This setting was preferred over using the standard leaf chamber with transparent top, in order to avoid any kind of shading of the leaf area caused by the frame and angle of measurement.

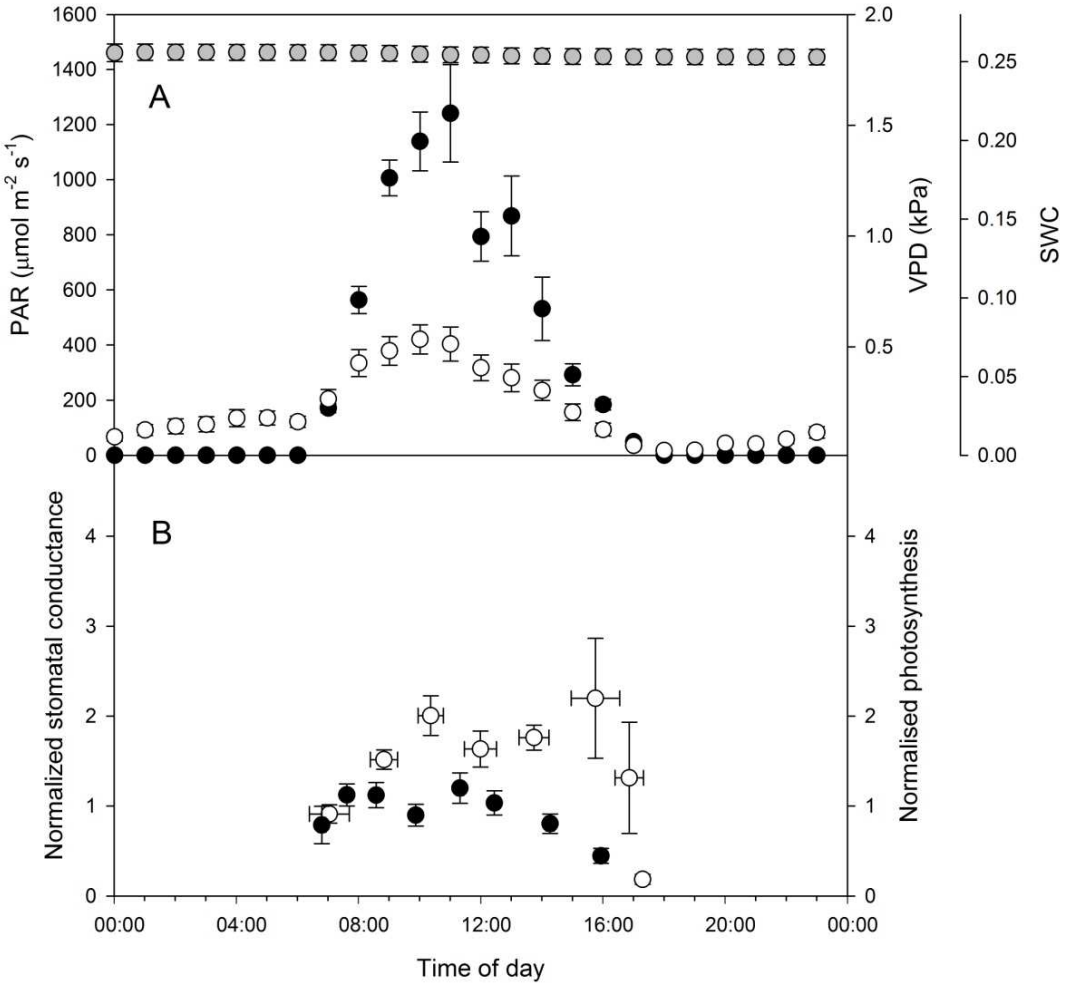

Figure 1. a) Average diurnal values of soil water content at 30 cm (SWC), vapour pressure deficit (VPD) and photosynthetically active radiation (PAR) ± SE for the days included in this study (n =12). Grey circles denote SWC, white circles VPD and black circles denote PAR. b) Average diurnal normalised photosynthesis (black circles, n =20) and stomatal conductance (white circles, n = ~100) ± SE and their time of measurement ± SE. The data for each species was normalised per species per sampling date.

4.2.4 Data analysis

Basic statistical analyses were performed in Minitab v. 15.1.1. In order to test for differences between 'sun' and 'shaded' leaves in *A* and g_s, paired t-tests were performed. To analyse the relationship between the environmental variables (PAR,

SWC, T_{air} , T_{leaf} and VPD), Ψ_{leaf} and g_s, stepwise regressions were performed. For this latter analysis, the meteorological data from the point in time at which the data was logged (every 10 minutes) that was closest to the time the Ψ_{leaf} and/or g_s were measured were used.

4.3 Results

4.3.1 Meteorology

The average VPD and PAR differed between the sampling days, although for their average daily values, only 23August 2008 had a significantly lower VPD ($P<0.05$) compared with the other days this study was conducted. No rainfall was observed throughout the study period. The average diurnal changes in VPD, SWC and PAR for all 12 measurement dates are shown in Figure 1, accompanied by normalized average diurnal *A* and g_s. The average daily SWC, VPD, PAR and T_{air} for two months of the dry season of 2008 that included the research period are given in Figure 2 and showed that the research days fell within the variance of this dry season. SWC ranged from 0.23 to 0.28 at 30 cm depth and between 0.14 and 0.16 at 1 m depth amongst the research days, while no distinct diurnal pattern of SWC was observed (Figure 1).

4.3.2 Average g_s, Ψ_{leaf} and A

All four species in this study had a similar between-leaves coefficient of variance (CV) for g_s (Table 1), showing that when measuring g_s at random for any of the four species, the variance from the mean is 40-50%. The within-leaves CV for g_s was relatively large for the species *Clusia cretosa* (Table 1). The average Ψ_{leaf} for both *Clusia cretosa* and *Schefflera allocotantha* are high compared with in *Weinmannia crassifolia* and *Clethra cuneata* (Table 2), and when all four species were measured at the same day, the Ψ_{leaf} values for *Clusia cretosa* and *Schefflera allocotantha* were equally high and did not change throughout the day, unlike the other two species (Figure 3). As for the g_s, when measured on the same day, no apparent differences in the range of observed g_s could be detected amongst the measured species, though on

the 8 of august, the average g_s from *Weinmannia crassifolia* seems lower than the other three species (Figure 3).

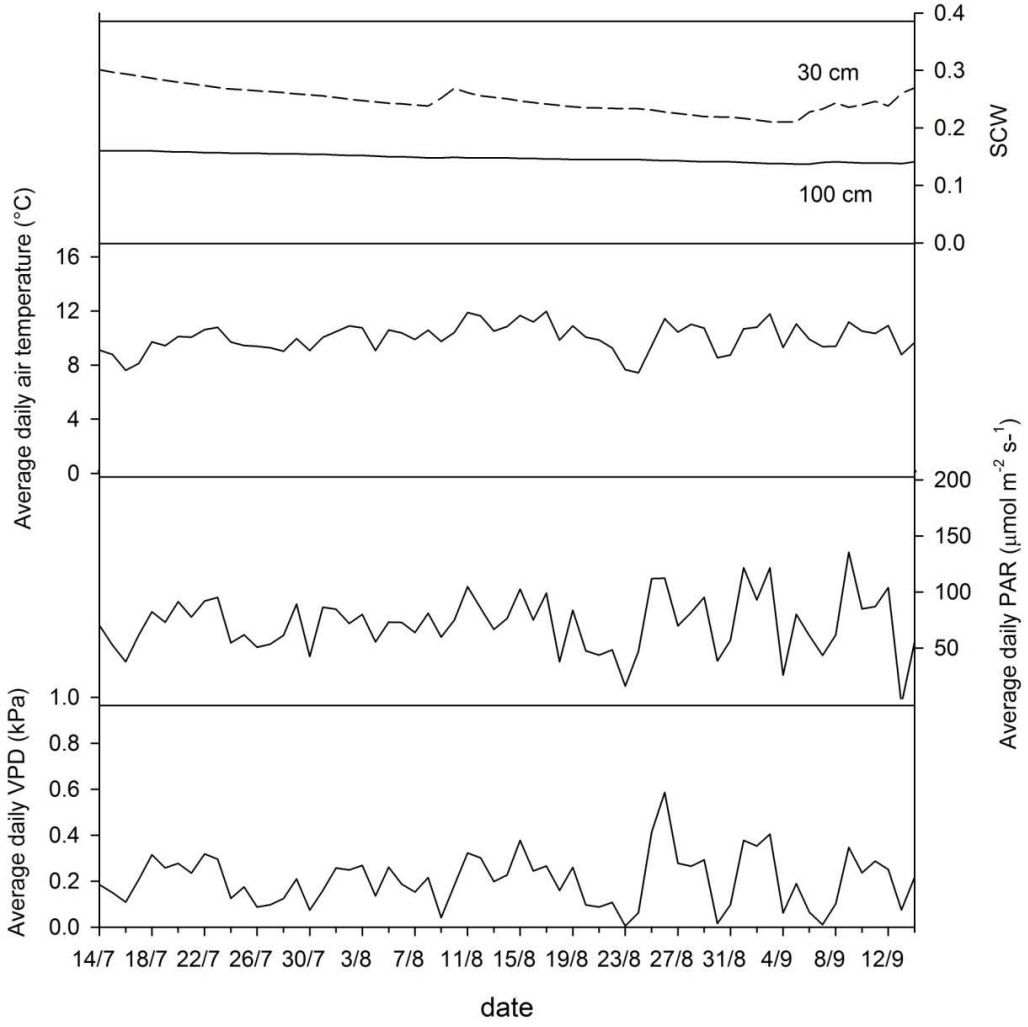

Figure 2. Average soil water content at 30 and 100 cm depth, daily temperature, photosynthetically active radiation (PAR) and vapour pressure deficit (VPD) between 14 July 2008 and 14 September 2008.

When comparing the g_s averages for all sampling dates per species, the average *Weinmannia crassifolia and Clusia cretosa* values are lower (Table 2, Figure 4). However, the overall average g_s values are the result of different sampling dates, making them harder to compare since the PAR and VPD differed amongst sampling

dates (Figure 2 and 4). Similarly, the average *A* values differed per species (Table 2, figure 5), but they were all four measured at different dates as well.

For all the investigated species, the paired t-tests showed that 'sun' leaves were receiving significantly more PAR compared to 'shade' leaves, both on days when *A* and g_s were measured ($P<0.04$, data not shown). 'Sun' leaves had significant higher values of *A* than 'shade' leaves for all four species ($P<0.05$), but no significant difference in g_s was found between the 'sun and 'shade' leaves ($P>0.11$) (Figure 5).

Table 1. The coefficient of variance (CV, variance divided by mean value) in stomatal conductance of 50 leaves per species and for five different positions per leaf for five leaves per species (%).

		Weinmannia crassifolia	*Schefflera allocotantha*	*Clusia cretosa*	*Clethra cuneata*
CV between 50 leaves		43.5	42.5	49.3	46.1
CV in leaf	Leaf 1	18.7	21.4	15.7	31.3
	Leaf 2	11.7	18.3	44.4	18.7
	Leaf 3	9.6	16.8	74.1	12.4
	Leaf 4	9.6	22.6	47.4	36.2
	Leaf 5	12.6	15.9	39.5	12.0
Average CV per leaf		12.4	19.0	44.2	22.1

Table 2. Number of observations (n), daytime averages (± SE), and minimum observed values for leaf water potential (Ψ_{leaf}), and maximum observed values for stomatal conductance (g_s) and photosynthesis (A) per species.

		Weinmannia crassifolia	*Schefflera allocotantha*	*Clusia cretosa*	*Clethra cuneata*
Daytime average (8:00–16:00)	*n*				
Ψ_{leaf} (MPa)	25	–0.695 ± 0.05	–0.002 ± 0.01	–0.019 ± 0.004	–0.580 ± 0.06
g_s (mmol m^{-2} s^{-1})	250–350	116 ± 5.2	216 ± 9.6	155 ± 9.7	265± 9.8
A (µmol m^{-2} s^{-1})	30	3.26 ± 0.2	5.16 ± 0.5	4.01 ±0.4	7.82 ± 0.4
Maximum or minimum observed					
Ψ_{leaf} (MPa)		–1.16	–0.021	–0.023	–1.57
g_s (mmol m^{-2} s^{-1})		489	749	780	720
A (µmol m^{-2} s^{-1})		7.2	10.8	8.71	12.9

4.3.3 Diurnal patterns

All four species from this study showed distinct diurnal patterns for both g_s (Figure 1, 3 and 4) and A (Figure 1), while for only *Weinmannia crassifolia* and *Clethra cuneata* Ψ_{leaf} changed throughout the day (Figure 3). Although for all species on August 10 the g_s decreased between 9:00 am and noon (Figure 3), on average both A and g showed no patterns of midday depression. The mean or normalised values of both g_s and A did not decrease around or after solar noon, or with increasing VPD and PAR (Figure 1 and 4). However, the four species did have different patterns in g_s at the end of the day. *Clusia cretosa* and *Clethra cuneata* both decreased towards the end of the day with decreasing PAR and VPD, unlike *Weinmannia crassifolia* and *Schefflera allocotantha*, for which g_s did not decrease around 16:00 (Figure 4).

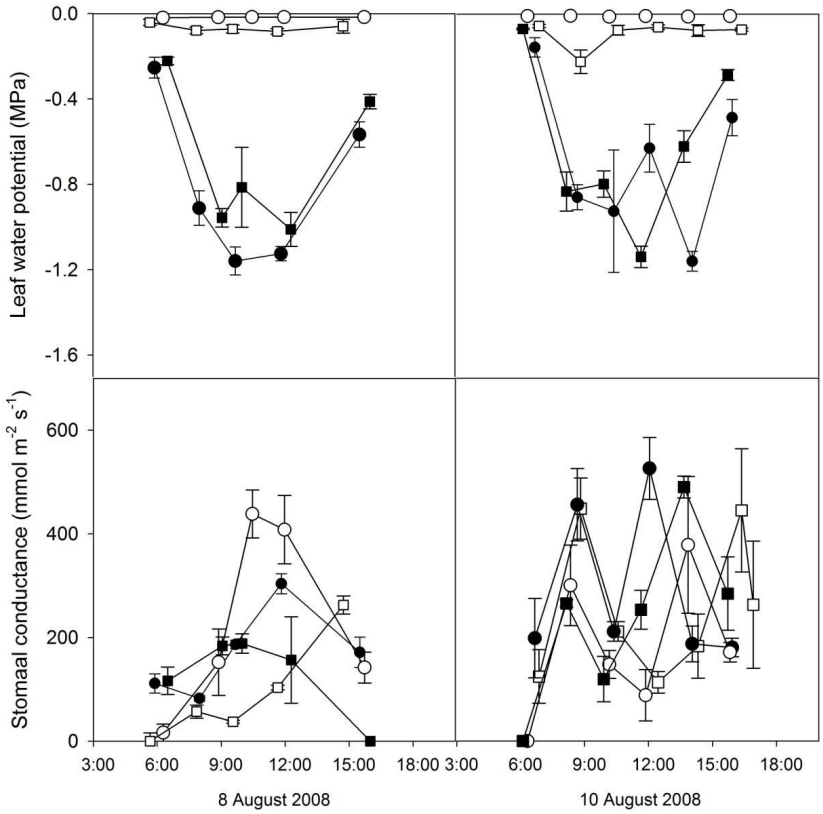

Figure 3. Diurnal changes in leaf water potential and stomatal conductance ± SE (n = 5) for the four investigated species on 8 and 10 August 2010. Black squares indicate *Weinmannia crassifolia,* white squares *Schefflera allocotantha,* black circles *Clethra cuneata,* and white circles *Clusia cretosa*.

4.3.4 Relationships between measured variables

When analysing the total dataset of g_s measurements with observed environmental variables (PAR, VPD, SWC, T_{air} and T_{leaf}), a stepwise regression showed a significant, but poor fit ($P<0.001$, $R^2=0.026$) of g_s with VPD, that improved only slightly when adding T_{air} to the regression equation ($P<0.001$, $R^2 = 0.089$), while including PAR, SWC or T_{leaf} did not improve the regression significantly (P=0.867, P=0.635 and P=0.564, respectively). The regression coefficient for VPD was negative, but for T_{air} it was positive. When performing the same analysis per species, *Weinmannia crassifolia* and *Clethra cuneata* did not show any significant relationships with any of the variables tested for.

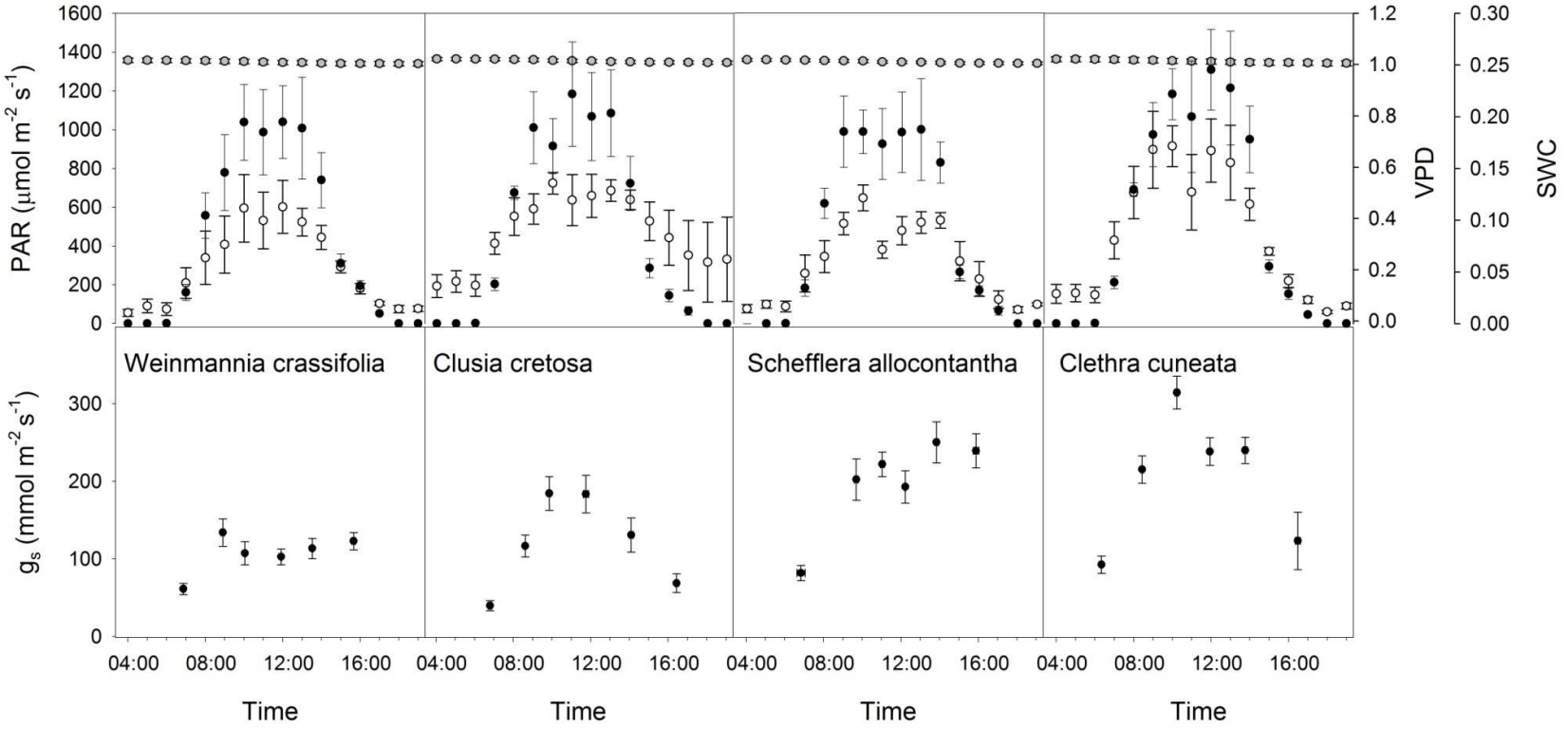

Figure 4. The diurnal changes in stomatal conductance (g_s) ± SE (n = 40-70) averaged for 5 different sampling days for the four investigated species (bottom panels), together with the accompanying average diurnal PAR (black circles), SWC (grey circles) and VPD (white circles) ± SE (n = 5) from the 5 sampling dates per species (upper panels)

For *Clusia cretosa*, the stepwise regression showed a positive significant regression with PAR ($P<0.001$, $R^2 = 0.19$), which was also observed for *Schefflera allocotantha* ($P<0.001$, $R^2 = 0.18$) and for these two species the regression did not improve by including VPD, SWC, T_{air} or T_{leaf}. When conducting the stepwise regressions including Ψ_{leaf}, no significant regressions were found for the any of the fours species, although when Ψ_{leaf} was added, these tests were performed on smaller datasets due to the smaller number of Ψ_{leaf} observations.

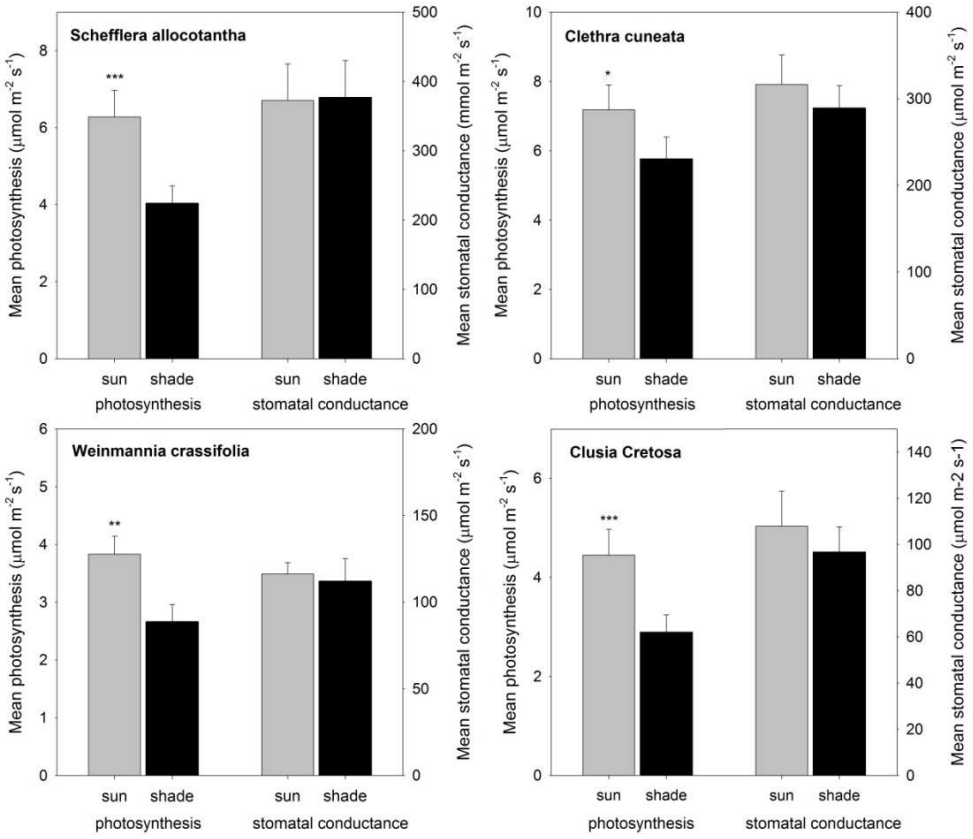

Figure 5. Mean daytime (8:00-16:00) photosynthesis (n = 30-48) and stomatal conductance (n =250-300) for fully sunlit and shaded leaves per species. Asterisks above the bars indicate the level of significance of the difference between fully sunlit and shaded leaves from a paired t-test (* = $P<0.05$, ** = $P<0.01$, and *** = $P<0.001$), while error bars indicate the SE of the mean. T_{leaf} ranged from 15.6-26.8 °C for these measurements.

4.4 Discussion

4.4.1 Leaf water status and stomatal conductance

The observed Ψ_{leaf} values (Table 2, Figure 3) were consistent with values from another TMCF and elfin forest in Jamaica and Venezuela (Kapos and Tanner 1985, Cavelier 1990), and slightly higher than observed in low montane cloud forests (Sobrado 2003). They were also higher than observed for lowland rainforest, in which values down to -4.3 MPa have been reported for mature trees in dry seasons, though most reported rainforest values are in between -1.5 and -2.0 MPa (*e.g.* Huc *et al.* 1994, Phillips *et al.* 2001, Brodribb and Holbrook 2004, Fisher *et al.* 2006). The four species included in this study had contrasting patterns for Ψ_{leaf} and g_s, with all species showing distinct diurnal changes for g_s, but both *Clusia cretosa* and *Schefflera allocotantha* had hardly any diurnal changes in Ψ_{leaf}. *Clusia cretosa* is known to be able to facultatively induce CAM metabolism in order to preserve water during its developmental stage. However, mature individuals of this species do not exhibit CAM metabolism under drought stress (Winter *et al.* 2008), and our g_s observations showed a C3 like pattern of stomatal conductance for this species, making it unlikely that CAM metabolism caused the Ψ_{leaf} to vary so little in this species.

The different patterns of g_s and Ψ_{leaf} amongst the four TMCF species could be a consequence of different stomatal 'strategies'. *Clusia cretosa* and *Schefflera allocotantha* both have g_s and Ψ_{leaf} patterns similar to isohydric species, while *Weinmannia crassifolia* and *Clethra cuneata* act anisohydrically. In other words, the former two species adjust their g_s to a level to an extent that Ψ_{leaf} does not change notably throughout the day, while for the latter two species both g_s and Ψ_{leaf} change throughout the day (*e.g.* Tardieu and Simonneau 1998, Mencucccini 2003, Franks et al, 2007). Throughout the measurement period, SWC decreased from 0.28 to 0.23 at 30 cm depth, but showed no diurnal variation. Equally, the pre-dawn Ψ_{leaf} values, which can be used as a proxy for soil water potential (*e.g.* Stričević and Čaki 1997), were not lower than -0.25 kPa at the measured days. This is lower than for example the pre-dawn dry season Ψ_{leaf} values measured in the cloud forest in Jamaica by

Kapos and Tanner (1985). Therefore, to properly test which of the four TMCF species from this study is an isohydric or anisohydric species, a larger range of soil water conditions should have been included in this dataset. However, since lower values of SWC did not occur throughout the measurement period, this was not possible. Additionally we have no data on root, xylem of leaf level ABA concentrations throughout the day. This makes it not possible to determine whether the roots of these TMCF species actually experience water stress at the given soil water conditions, and whether this is signal is transported from the roots to the leaves.

Alternatively, the lack of variance in diurnal Ψ_{leaf} for *Schefflera allocotantha* could be a consequence of lower leaf area of the selected trees. Although leaf area was not quantified in this study, the canopies, and hence total leaf area, were smaller for *Schefflera allocotantha* than for the other species. When plants have a lower leaf area but equal access to water, it potentially loses less water through transpiration on a canopy basis, resulting in smaller changes in the Ψ_{leaf} (Eavis and Taylor 1979, Vertessy *et al.* 1995). Furthermore, species with succulent leaves have a larger capacitance (Smith *et al.* 1987), and since *Clusia cretosa* has succulent leaves, the larger capacitance of this species can act as a buffer to cause the little diurnal variation of Ψ_{leaf}. Overall, observed Ψ_{leaf} was relatively high (Table 2), and never lower than -1.57 MPa, which was observed once at midday in *Clethra cuneata*, indicating that even though this study was carried out in the dry season, the vegetation was unlikely to experience drought stress similar to tropical lowland rainforests. Finally, *Weinmannia crassifolia* as *Clusia cretosa* are very abundant species in the studied area (Feely and Silman, in press), and the species included in this study together cover 48% of the research site. However, this does not exclude the possibility that there are species present at the research site that would have different patterns of Ψ_{leaf} and also g_s. Overall, the contrasting diurnal patterns of Ψ_{leaf} amongst the studied species emphasize the importance of not generalising the hydraulic behaviour of a forests, base in single species.

4.4.2 Diurnal stomatal conductance and controls

The observed rates of g_s (Table 2, Figure 3 and 4) did not differ from those observed in other TMCFs and elfin forests (Cavelier 1990, Letts and Mulligan 2005, Motzer *et al.* 2005), or those observed in tropical lowland rainforests (*e.g.* Santiago *et al.* 2000, Marenco *et al.* 2001, Brodribb and Holbrook 2004, Meinzer *et al.* 2008).This confirms the hypotheses of Bruijnzeel and Veneklaas (1998) that TMCF g_s is not inherently low. As for the diurnal patterns of g_s, none of the studied TMCF species experienced an apparent midday depression in g_s, unlike the species studied by Cavelier in Colombia (1990), which had reduced g_s values around noon at similar VPD values. However, as mentioned in the previous section, based on the pre-dawn Ψ_{leaf} values in the elfin forest of Cavelier (1990), the soil water availability might have been lower for the trees, resulting in a decrease of g_s throughout the day. Midday depression of g_s (and A) has also been observed amongst species in tropical lowland forests (Brodribb and Holbrook 2004, Ishida *et al.* 2005, Tay *et al.* 2007). Pons and Welschen (2003) showed experimentally that for some tropical leaves at temperatures below 33 °C, a decrease in g_s is a consequence of increased VPD is the principal reason for reductions in A. However, in the studied TMCF, the range of experienced VPD values was not as large as in the mentioned studies from other TMCFs or tropical rainforests. For example, in the study of Ishida *et al.* (2005) the top of the canopy leaves of a lowland rainforest in Indonesia experienced VPD values between 2-4 kPa. Therefore, the poorly fitting relationship between VPD and g_s in this study might be the effect of sampling g_s at a relatively small range of VPD values. It is therefore not certain what the relationship between g_s and VPD in the studied species would be at VPD values higher than observed, or at lower soil water availability.

Climate modelling exercises predict lower humidity and higher temperatures in the eastern tropical Andes towards the end of the 21st century (Vuille *et al.* 2003, Urrutia and Vuille 2009). This would translate in a higher range of experienced VPD by the studied TMCF and most likely to less soil water availability. Therefore, under anticipated climate change, both VPD and SWC will probably become a more important factor in controlling stomatal opening and closure of this TMCF.

However, under the current general ‘dry season’ circumstances, VPD, and soil water are unlikely to limit daytime g_s and it is more likely that other factors, like PAR and A drive g_s.

4.4.3 Photosynthesis

The range of A and the maximal A observed in the four species (Figure 3) are similar to those found in studies in Ecuador, Venezuela and Borneo (Hikosaka *et al.* 2002, Letts and Mulligan 2005), as well as those observed in tropical lowland forests (*e.g.* Carswell *et al.* 2000, Domingues *et al.* 2007, Meir *et al.* 2007), implying that on a leaf area basis, *in situ* C-uptake is not lower than in other tropical forests. This indicates that if TMCFs have a lower gross C-uptake compared with tropical lowland forests, the total amount of leaf area might be of more importance determining the difference in total canopy C-uptake than the inherent photosynthetic capacity of the leaves. Furthermore, since g_s was hardly sensitive to changes in VPD, diurnal changes in A are mostly driven by changes in PAR levels and diurnal temperature changes, rather than stomatal closure. This is confirmed as well by the lack of difference in g_s between ‘sun’ and ‘shade’ leaves (Figure 5). As mentioned above, this is not to say that g_s would not play a role in controlling daily C-uptake if atmospheric demand would be higher than observed in this study, or if the water availability would be lower. However, it merely indicates that for the observations made here, A was not limited by leaf or soil water status or VPD. In addition, Foster (2001) coined the (so far untested) hypothesis that TMCFs could experience low photosynthesis rates in the second half of the day, if under unclouded conditions in the morning the photosynthetic apparatus is damaged by the higher levels of UVB which plants at high altitudes receive. Although we did not investigate the influence of received UVB:PAR ratios on the studied trees experimentally, Figure 1 shows no lower rates of photosynthesis in the second half of the day under equal PAR levels, compared with the first part of the day. Therefore, we expect that limitation of A through the UVB damage does not play a role in this context.

4.5 Conclusions

The four investigated species had different diurnal patterns in Ψ_{leaf} and g_s, with *Clusia cretosa* and *Schefflera allocotantha* acting more like isohydric species, and *Weinmannia crassifolia* and *Clethra cuneata* like anisohydric species. However, the observed range in soil water status did not provide enough information to draw more definite conclusions on this. None of the four species experienced midday depression in g_s or A, and relationships between g_s and the measured environmental parameters were weak or absent. This implies that under the current dry season conditions, these four species do not experience drought stress to a level that it induces stomatal closure. Consequently, the C-uptake in this area is unlikely to be limited by soil water status or VPD under current circumstances, though this might change under anticipated climate change. Finally, the observed levels of A were similar to those found in tropical lowland forests, which indicates that on a leaf level basis, TMCF do not have a lower inherent photosynthetic capacity.

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Chapter 5

Paper 4: Evaluating the environmental controls on the GPP in a tropical montane cloud forest in Peru

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Abstract

The net primary productivity (NPP) of tropical montane cloud forests (TMCF) is known to be lower than in tropical lowland forests that occur at similar latitudes. However, estimates of gross primary productivity (GPP) of TMCFs are scarce, and there are uncertainties in what factors are most important in controlling TMCF productivity. In this study, the annual GPP of a TMCF at 2990 m a.s.l. in south east Peru was simulated by using a Soil-Plant-Atmosphere model developed by Williams (1996). It is the first attempt to model TMCF GPP that uses both physiological and structural parameters of the vegetation and soil collected in the field, combined with a one year meteorological data set. Simulated annual GPP was 16.2 ± SE 1.6 t C ha^{-1} yr^{-1}, which is about half of the GPP commonly observed in neotropical lowland rainforests. Analyses of the modelled results indicated that the variation in GPP in this TMCF is mostly controlled by the meteorological drivers air temperature (T_{air}) and photosynthetic active radiation (PAR), and the structural parameter leaf area index (LAI). This latter factor is only of importance if the photosynthetic capacity per leaf area stays constant. When T_{air}, PAR and LAI were increased to values reflecting the conditions of a tropical lowland forest, GPP increased up to 75%. Higher radiation levels increased GPP if the diffuse portion of irradiance stays the same. However, since increases in mean daily radiation at the research site are a consequence of less cloudiness, this also results in less diffuse radiation, and lower proportions of diffuse light decreased GPP at any given average irradiance level. Therefore, the cloudy condition of TMCFs should not be regarded simply as a negative control on TMCF GPP by reducing the total irradiance. Instead, the presence of clouds partially offsets the decrease in GPP following reductions in radiation. Furthermore, the modelled results indicated that the TMCF in this study is very insensitive to changes in the hydraulic parameters, or changes in soil water content, which provided more evidence that, under current conditions, (periodic) drought stress is a very unlikely explanation for the lower TMCF GPP.

Keywords: SPA model, Peru, tropical montane cloud forest, GPP, diffuse radiation

5.1 Introduction

Tropical montane cloud forests (TMCF) differ from lowland rain forests, both in their structure and functioning. In general, tree stature is smaller, leaves have a higher leaf mass per area (LMA), and leaf area index (LAI) is lower (*e.g.* Grubb and Whitmore 1966, Grubb 1977, Vitousek *et al.* 1992, Lieberman *et al.* 1996, Raich *et al.* 1997, Tanner *et al.* 1998, Kitayama and Aiba 2002, Moser *et al.* 2007, van de Weg *et al.* 2009). The gross primary productivity (GPP) of TMCFs has for a long time been thought to be lower than tropical lowland rainforests, which is mainly based on the observation of smaller amounts of litter fall and lower rates of trunk diameter increments that indicate reductions in aboveground net primary productivity (ANPP) (Weaver and Murphy 1990, Raich *et al.* 1997, Herbert and Fownes 1999, Kitayama and Aiba 2002). Suggested explanations for the lower ANPP of TMCFs include low levels of photosynthetic active radiation (PAR) through frequent cloud events, low temperatures, low nutrient availability limiting growth and photosynthetic capacity, periodic water deficiencies causing stomatal closure, and leaf wetness inhibiting photosynthesis and reducing the plant surface temperatures (Grubb 1977, Bruijnzeel and Veneklaas 1998, Waide *et al.* 1998, Letts and Mulligan 2005, Soethe *et al.* 2008). Some nutrient addition experiments have been conducted in order to test the hypotheses concerning nutrient limitation in TMCFs. Although N and/or P addition increased stem growth and litter fall in some studies (Tanner *et al.* 1990, Tanner *et al.* 1992, Vitousek and Farrington 1997), not all TMCF species increased their ANPP after nutrient addition in other nutrient addition experiments (Cavelier *et al.* 2000, Adamek *et al.* 2009). Furthermore, N and/or P addition did not alter the foliar nutrient content in all TMCF nutrient addition experiments, leaving it unclear as to whether nutrient addition actually increased overall carbon (C) uptake or alternatively changed C allocation between aboveground and belowground components. Most other plausible explanations for the lower ANPP of TMCFs, for example low levels of PAR, water logged soil conditions or lower temperatures (Bruijnzeel and Veneklaas 1998, Letts and Mulligan 2005), remain untested with either experiments or modelled simulations.

Extending the notion of radiation-driven constraints on ANPP, even though frequent cloud events are a key feature of TMCFs (Stadtmüller 1987), the role of diffuse radiation on GPP in TMCFs resulting from this cloudiness has remained unexplored. This is remarkable, since the influence of diffuse radiation on canopy C-uptake has been noted repeatedly over the last decade (*e.g.* Roderick *et al.* 2001, Alton *et al.* 2007, Knohl and Baldocchi 2008, Mercado *et al.* 2009). Letts and Mulligan (2005) argued that since for some tropical lowland rainforests an increase in diffuse light on cloudy days does not compensate fully for the reduction in PAR (Graham *et al.* 2003), TMCF GPP would also not be enhanced through increases in diffuse light. However, whether or not a large fraction of diffuse light increases GPP has proven to depend on many factors including structural variables like LAI and the spatial and angular distribution of leaves (Alton *et al.* 2007, Knohl and Baldocchi 2008), together with concurrent meteorological changes in air temperature and relative humidity (Urban *et al.* 2007, Min and Wang 2008, Wohlfahrt *et al.* 2008). These factors differ in TMCFs compared to other forests, including tropical lowland rainforests, which makes it difficult to predict the relationship between diffuse radiation and GPP in a TMCF from relationships observed in other biomes.

In addition, recent findings on the NPP of TMCFs showed that the low ANPP values generally observed do not necessarily have to correspond to an equal decline in GPP. The aboveground/belowground ratio in carbon allocation appears to be substantially smaller in TMCFs than in lowland rainforest, meaning that in TMCFs, more C is allocated to fine root biomass (Roderstein *et al.* 2005, Leuschner *et al.* 2007) and soil organic C (Zimmermann *et al.* 2009). Although lower temperatures at higher altitudes partially explain the higher residence time in fine root biomass (Girardin *et al.* in press), total C-input rates have hardly been estimated for TMCFs, leaving it unclear whether the higher C stock in the TMCF soil is solely the result of a lower C-return rate, or if relatively higher GPP values are important as well. The very few studies on leaf-level photosynthetic rates in TMCFs suggests that the photosynthetic capacity of TMCF leaves is not systematically lower than in lowland rainforests when expressed on an area basis (Hikosaka *et al.* 2002, Letts and Mulligan 2005). These findings therefore suggest that although NPP is lower in TMCFs compared to

lowland tropical rainforest (Girardin *et al.*, in press), this does not inevitably correspond to a proportionally lower GPP.

No direct estimates of TMCF C-uptake are available in the literature to our knowledge, perhaps because direct measurement of CO_2 exchange between vegetation with the atmosphere using the eddy covariance technique is difficult in mountainous terrain (Kaimal and Finnigan 1994). Alternatively, an estimation of annual GPP can be made by summing all the respiration and growth components of a forest ecosystem (Malhi *et al.* 2009). This approach requires the assumptions that carbon storage by trees does not fluctuate between years or seasons, which is unlikely based on observations in the field (Würth *et al.* 2005), and/or that all important respiration and growth components can be measured with adequate precision. Alternatively, GPP can be estimated using weather data and a canopy model specified with site-based measurements; such an estimate of GPP could also be used to test GPP estimates made by other means. In this study we simulated the GPP of a TMCF in Peru by using the Soil-Plant-Atmosphere (SPA) model developed by Williams *et al.* (1996). To our knowledge, this is the first modelled estimate of TMCF GPP that uses photosynthetic parameters that were measured *in situ*. Furthermore, we investigated how TMCF GPP is controlled by environmental conditions and canopy structure, as informed by the most recently validated version of SPA (Fisher *et al.* 2007, Fox *et al.* 2009). The research questions for this study were: 1) What is the estimated GPP of a TMCF using physiological and structural parameters measured in the field and a Soil-Plant-Atmosphere model? 2) What are the key environmental controls on TMCF GPP, and to what extent do they explain the difference in TMCF and lowland tropical rainforest productivity? 3) How does the ratio of diffuse and direct light affect TMCF GPP?

5.1.1 Rationale for choosing the SPA model

The SPA model was chosen because it is a multilayer soil-vegetation–atmosphere-transfer model (SVAT), which provides a simulation of C3-canopy processes (Williams *et al.* 1996). It operates at a 30-min time step, and incorporates 10 canopy

layers and 20 soil layers (Williams *et al.* 2001b). The radiative transfer scheme in the model simulates absorption and scattering of direct and diffuse PAR, near infra-red radiation (NIR) and long wave radiation in all canopy layers. This detail provides a fine spatial and temporal scale suitable to model the consequences of the diurnal changes in microclimate and physiology of a canopy. C-uptake is modelled by linking the well tested Farquhar equation for leaf-level photosynthesis (Farquhar and Von Caemmerer 1982) with the Penman-Monteith equation to determine leaf-level transpiration (Jones 1992), by a model of stomatal conductance (g_s) that optimizes daily C gain within the limitations of canopy water storage and soil-to-canopy water transport. SPA models soil-to-leaf water transport and provides an explicit link between canopy structure, soil properties and soil moisture, atmospheric conditions and stomatal conductance. Species are not simulated individually; instead the vegetation is treated as a single functional type, with vertical variation in Rubisco carboxylation efficiency (V_{cmax}), electron transport efficiency (J_{max}), light absorbing leaf area (LAI), and plant hydraulic properties. The model has performed well in modelling a variety of ecosystems, from Arctic tundra to Amazonian rainforests; where fully quantified, the error in the output from SPA has been less than 10% (Williams *et al.* 1996, Williams *et al.* 1998, Williams *et al.* 2001a, Williams *et al.* 2001b, Fisher *et al.* 2007, Fox *et al.* 2009). The model simulated GPP and measurements of stomatal conductance, sap flow and soil water availability from an Amazonian lowland rainforests particularly well (Fisher *et al.* 2006, Fisher *et al.* 2008). Given these results and because the validation data available to us consisted of a sap flow data set, the SPA model is well suited for this modelling exercise

5.2 Methods

5.2.1 Fieldwork site

The study was performed in a previously established 1 ha plot in a tropical montane cloud forest located in the Kosñipata valley in south east Peru at 2990 m a.s.l. (13º11’28’’S / 71º35’24’’W), near the Wayquecha research station. The research site has an easterly aspect, with a 27.67 % slope, a mean annual T_{air} of 12.5 ± SE 0.05 ºC,

and mean annual rainfall ranges between 1700 and 2000 mm yr^{-1}. The forest at the research site is dominated by species in the *Weinmannia* and *Clusia* genera. The average canopy height is 12.8 ± SE 0.46 m (n = 180) and average soil depth is 0.44 ± SE 0.06 m (n =10).

5.2.2 Meteorology

An automated weather station (Campbell Scientific Ltd, UK) was positioned at the edge of the experimental plot, and data was collected every 10 seconds and stored as 10 minute averages using a data logger (CR3000, Campbell Scientific Ltd, UK). Precipitation was measured with a tipping bucket rainfall gauge with a 0.2 mm resolution, together with a two fog collectors (harp and mesh) with a similar resolution (Campbell Scientific Ltd, UK). Photosynthetically active radiation (PAR) was measured with a PAR quantum sensor (Skye Instruments Ltd, Powys, UK), while a net radiometer measured short and long wave radiation (CNR1, Kipp & Zonen, Delft, Netherlands) and the diffuse radiation was registered with a sunshine sensor (BF3, Delta-T Devices Ltd, Cambridge, UK). Air temperature and relative humidity were measured with a combined HMT sensor (Vaisala, Oy, Finland), and vapour pressure deficit (VPD) was calculated from that data by the data logger of the weather station (CR3000, Campbell Scientific Ltd, U.K.).

Meteorological data were missing (due to battery failure of the data logger) from 27 February 2009 to 3 March 2009 and for 30 April 2009, representing less than 2% of the total data set. Data for these two gaps were filled for all the meteorological values, using a mean monthly diurnal cycle, with the missing period being centred in that month. For short wave radiation, data were missing from 19 September 2008 to 9 December 2008, representing 22% of the short wave radiation dataset. These gaps were filled by recalculating the total short wave radiation from the collected PAR data in that period, using a regression for each daily 30 minute time step of the model. The average coefficient of variation (CV) for the transformation factors was 0.24. A similar procedure was carried out to calculate the diffuse and direct part of the long wave radiation for this period (CV = 0.27).

5.2.3 Sap flux

Sap flow rates were measured for 12 trees located well within the research plot in order to validate the modelled results. The species included in the sap flux measurement were *Clusia cretosa* (5 trees), *Weinmannia crassifolia* (3 trees), *Prunus integrifolia* (1 tree), *Clusia flavifora* (1 tree), *Weinmannia bangii* (1 tree), and *Clethra* sp. (1 tree). Sap flow sensors were installed in April-May 2007 with trees selected to represent the diameter distribution present in the plot (12.95-38.07 cm). Sap flow was measured by the trunk segment heat balance method described by Cermak *et al.* (1973, 2004) (Sap Flux Meter P4.1, Environmental Measuring Systems, Brno, Czech Republic). Sap flux in each tree was measured every minute and averaged over every 10 min period throughout each day. Due to limited power supply throughout the year, the data collection period was 16 July 2008 to August 2008, with five days missing because of power failure. The method does not require calibration for xylem depth if the sensors (30-50 mm long) cover all the active xylem tissue horizontally. Xylem depth was estimated by coring individual trees using a Suunto (Finland) increment borer. Cores were taken from 85 trees ranging from 10-44.9 cm in diameter at breast height (DBH) approximately 500 m away from the research plot (in order to avoid disturbance of other research activities). Active sapwood depth, identified by observation and using dye, showed a tight significant linear relation with tree diameter ($R^2 = 0.78$, $P < 0.01$, $n = 85$), and the observed relationship suggests that electrodes inserted to 20 mm depth adequately sampled all the conductive xylem in all the trees of the study (DBH $\leq$ 38.07 cm, estimated active xylem dept $\leq$ 16.0 mm). Average sap flux density per cm tree circumference (J_s, kg hr^{-1} cm^{-2}) was not significantly correlated with tree diameter ($P = 0.32$, $R^2 = 0.11$, $n = 12$), so tree diameter was not used to scale sap flow to the stand-scale. Instead, the average sap flow per unit of circumference was scaled by tree size across all trees in the plot to estimate water use on a ground area basis.

Table 1. Photosynthetic parameters V_{cmax} and J_{max} on an area basis and corrected to the temperature optimum of 30 °C (as embedded in the SPA model), together with the proportion of the leaves per canopy layer as represented in the standard run of the SPA model. The average V_{cmax} and J_{max} are based on the proportional representation of the measured species in the research plot.

Canopy layer (1= top canopy)	**V_{cmax} (μmol m^{-2} s^{-1})**	**J_{max} (μmol m^{-2} s^{-1})**	**Proportion of leaves per layer**
1	45.9	95.2	0.203
2	34.6	81.2	0.350
3	28.3	62.5	0.214
4	23.2	50.9	0.143
6	12.8	29.1	0.052
7	15.4	30.6	0.017
8	15.1	29.9	0.011
9	-	-	-
10	-	-	-

5.2.4 Photosynthetic parameters

The photosynthetic parameters V_{cmax} and J_{max} were measured by deriving A-C_i curves of the 5 most abundant species throughout the canopy, using portable photosynthesis equipment (Li-Cor 6400, Li-Cor, Inc, Lincoln, USA), fitted with a LED light source (6400-02B Red/Blue Light Source) according to the procedural guidelines in Long and Bernacchi (2003). In total, 78 leaves were measured at the top, middle and bottom of the canopy. The fits were obtained using the Farquhar biochemical model of leaf photosynthesis (Farquhar *et al.* 1980, von Caemmerer and Quick 2000), with a modification for TPU by Harley *et al.* (1992). The enzymatic kinetic constants were taken from von Caemmerer (2000), assuming an infinite internal conductance term, as examined by Domingues *et al.* (2010). The curve fitting was based on minimum least-squares using a routine developed for use in the "R" environment (R Development Core Team, 2008). The average V_{cmax} and J_{max} used in the model were

based on the basal area each measured species represented in the 1 ha research plot. The A-C_i curves were performed at ± 20 °C and adjusted to 30 °C according to the temperature response curve embedded in the SPA model.

5.2.5 Structural vegetation characteristics

Leaf area index (LAI) was obtained using two different methods. On 14-15 July 2008, the gap fraction of the canopy was estimated using a hand-held spherical densitometer (Lemmon 1956) at 180 locations equally distributed in the 1 ha research plot. LAI was calculated from these numbers using the Beer-Lambert equation with a spherical light extinction factor for broad leaf trees (k=0.5). For the changes in LAI throughout the year, we used the annual variability in LAI measured by Metcalfe *et al.* (in press) using the hemispherical photographic method. The fraction of the total LAI per canopy layer (n = 10) was estimated in the field from the trees of which the V_{cmax} and J_{max} values were measured (n =78) (Table 1). A pressure chamber was used to measure the minimum leaf water potential (Ψ_{leaf}) of sample trees just outside the research plot and the minimum observed value was -1.56 MPa. Root biomass values were adapted from Girardin *et al.* (in press) as well as root density and root biomass density.

Table 2. Structural parameters and their units used in the standard SPA model simulations and their origins.

Parameter	Units	Value	Source
Canopy height	m	12.8	Estimate from field
Aboveground conductance	m^2 MPa $mmol^{-1}$	3.5	Parameterised according to leaf specific conductivity (*LSC*)
Root resistivity	MPa s g $mmol^{-1}$	140	Parameterised according to leaf specific conductivity (*LSC*)
Iota	none	0.0007	Set
Rooting depth	m	0.3	Measured in field
Capacitance	mmol MPa^{-1} m^{-2}	5000	Set

5.2.6 Soil characteristics

Soil depth in the experimental plot was measured in 2008 at 10 randomly-selected locations in the hectare plot and the average soil depth (0.44 ± SE 0.063 m) and observed rooting depth of fine roots was up to 30 cm (C. Girardin, pers. comm.). Soil properties (organic fraction, sand and clay fraction of the soil) were adapted from Zimmermann *et al.* (2009).

5.2.7 Modelling methodology

A detailed description of the SPA model can be found in Williams *et al.* (1996). In order to avoid duplication, here we only address the aspects that are important for the research questions that were posed regarding the importance of radiation in controlling TMCF GPP. Foliar C-uptake in the SPA model is based on the Farquhar equation for C3 photosynthesis (Farquhar and Von Caemmerer 1982) and the photosynthetic parameters representing the carboxylation efficiency (V_{cmax}) and electron transport (J_{max}) are based on foliar N concentrations (Field and Mooney 1986) and leaf temperature, according to the following equations:

$$V_{cmax} = N\ \tau_c\ \kappa_c$$

and

$$J_{max} = N\ \tau_j\ \kappa_j$$

where N is leaf nitrogen content (g m^{-2}), and τ_c and τ_j are the respective temperature coefficients and κ_c and κ_j are the are the respective catalytic rate coefficients for V_{cmax} and J_{max}. For this modelling exercise, leaf N was adapted to represent the V_{cmax} and J_{max} values as measured *in situ*.

The model requires measured PAR values as an input, as well as the diffuse fraction of radiation and short wave radiation. A subroutine calculates sun-lit and shaded fractions of the foliage for each time step (30 minutes) in each of the 10 canopy

layers whilst the absorption of PAR in each canopy layer is determined by a Beer-Lambert assumption. Each sun-lit foliage fraction is lit by both incident direct PAR, which is the same as the incident direct radiation at the top of the canopy layer, and the diffuse PAR received by that layer. The shaded fraction for the same layer receives only diffuse PAR that penetrates to that layer. The reflectivity and transmissivity of leaves are estimated from empirical data (Baldocchi and Harley 1995). After the first downward pass, there is an upward pass through the canopy to determine the fate of the reflected radiation and any beam of radiation that is reflected or transmitted is converted to diffuse radiation. Radiative transfer of NIR follows the same principles as for PAR, but there are different reflectance/transmittance constants, and no separation of sunlit and shaded leaf fractions. A spherical leaf angle distribution is assumed (Russell *et al.* 1989), so the proportion (P) of radiation incident on the top layer that passes through without striking a leaf is given by

$$P = \exp(-GL_i/\sin\beta)$$

where G is 0.5 for a spherical leaf angle distribution, L_i is the leaf area ($m^2\ m^{-2}$) in canopy layer i, and β is the elevation of the radiation source (which varies with latitude and time of day for beam radiation (Jones 1992); for diffuse radiation it is a constant 30°). Temperature sensitivity of photosynthesis is represented in both the calculation of V_{cmax} and J_{max} and they are described using response curves (Rastetter *et al.* 1991) fitted to the polynomial relationships of McMurtrie *et al.* (1992), with temperature optima set to 30°C.

5.2.8 Aboveground and belowground parameterization

The non-photosynthetic parameters used by SPA are listed in Table 2. The above- and belowground hydraulic resistance (i.e. stem conductivity and root resistivity) were calibrated with leaf specific conductance (*LSC*, mmol $m^{-2}\ s^{-1}\ MPa^{-1}$). Aboveground and belowground resistance were assumed to be approximately equal. *LSC* was calculated with independent transpiration (E, mmol $m^{-2}\ s^{-1}$) and water leaf

potential measurements (Ψ, MPa) made from 2 to 5 August 2008 on the same species that were selected for sap flow measurements, assuming that

$$\Delta\Psi = \Psi_{leaf} - \Psi_{predawn}$$

and

$$LSC = E/\Delta\Psi$$

The average *LSC* was 1.74 mmol m^{-2} s^{-1} MPa^{-1}. The parameter determining the incremental increase in photosynthesis necessary for stomata to open, iota (ι, dimensionless), was set as 0.0007, to give a maximum stomatal conductance (g_s) of 0.5 mol m^{-2} s^{-1}, to approximate maximum values of g_s observed in the field. In SPA, g_s is incremented until it fails to increase C-uptake by more than 1-ι. Since the simulated TMCF was not expected to be drought limited, we used the standard low input of ι = 0.0007. Capacitance of the simulated forest was set at 5000 mmol MPa^{-1} m^{-2}, which matched the tails of hourly sap flow in the field. Soil porosity was calculated with the organic fraction, sand fraction, and clay fraction according to Saxton *et al.* (1986).

5.2.9 Sensitivity analyses

Sensitivity analyses were performed to investigate what parameters are most important in controlling the GPP in the simulated TMCF. A previous analysis using SPA identified 9 factors that explain the global variation in daily variation of total canopy C-uptake (Williams, 1998), with LAI and total irradiance being the most important. For the sensitivity analyses, each factor was changed individually across the range observed in the field, keeping daily and seasonal variation proportional to the original observed values, while the others were kept constant. For sensitivity ranges, all daily minimum and maximum average values observed in the field for T_{air}, VPD, short wave radiation, and PAR were used (Table 3). For LAI, the minimum and maximum measured values in the research plot were used.

Table 3. The units and standard values for the parameters used to run the SPA model, and their maximum and minimum values as measured in the field used for the sensitivity analyses. From top to bottom, the table shows the parameters to which the simulated GPP was most sensitive, to the least sensitive. For V_{cmax} and J_{max}, only the values from the top canopy layer are shown, though the values at the other canopy layers were changed accordingly for the sensitivity analyses. The maximum and minimum plant conductivity and root resistivity were parameterized according to maximum and minimum observed leaf specific conductance (*LSC*). For iota and capacitance the table presents the value for which the sensitivity analysis was performed, though no field observations were available for these factors..

Factor	**Unit**	**standard**	**maximum**	**minimum**
V_{cmax} - J_{max}	µmol m^{-2} s^{-1}	40.1-76.1	93.9-140	14.9-26
Average daily PAR	µmol m^{-2} s^{-1}	233	500	46
Average daily shortwave radiation	MJ m^{-2} d^{-1}	8.765	20.3	1.76
LAI (N in leaves stays constant)	-	4.17	5.6	2.6
Average daily temperature	ºC	10.44	13.26	7.34
Fraction diffuse light	-	0.42	0.5	0.2
Average daily VPD kPa	kPa	0.14	0.36	0.03
LAI (N in canopy constant	-	4.17	5.6	2.6
Total root biomass m^{-2}	g	3291	5420	1967
Root resistivity	MPa s g $mmol^{-1}$	145	180	120
Plant conductivity	mmol m^{-1} s^{-1} MPa^{-1}	3.5	4.4	2.75
Soil water content		0.29	0.367	0.196
Iota (ι)	-	0.0007	0.001	0.0001
Capacitance	mmol m^{-2} LA MPa^{-1}	5000	3000	7000

The photosynthetic parameters V_{cmax} and J_{max} were changed by hanging the SPA catalytic coefficients κ_c and κ_j simultaneously. Since under field conditions both parameters are highly correlated (Wullschleger 1993), they were not tested separately.

For the aboveground and belowground hydraulic resistivity, we used the minimum and maximum measured *LSC* to calculate this. No effects on GPP were found when changing *LSC* within the ranges observed, therefore we additionally tested what minimum or maximum values of above and belowground resistivity would cause the annual GPP to decline. No measurements of capacitance, or ι were performed, so we tested the sensitivity to changes of capacitance between 3000 and 7000 MPa^{-1} m^{-2}, and ι between 0.001 and 0.0001, but GPP was insensitive to these ranges. Furthermore, we tested the sensitivity of GPP to different values of soil water content (SWC). We used fixed values, since daily variation was not possible to include. First within the range of the maximum and minimum observed values (SWC = 0.196-0.367 m^3 m^{-3}), but also to a lower minimum, since GPP did not decrease with the lower observed SWC.

In addition to the one dimensional sensitivity analysis, a more specific analysis was carried out to test the importance of direct and indirect radiation on TMCF GPP. For a range of mean daily PAR levels, annual GPP was simulated at different ratios of (daytime) diffuse light, keeping all other factors constant (though long wave radiation changed concordantly with PAR) and the daily and seasonal variation proportional to the original observed values. Additionally, for each of the different ratios of diffuse light, the average PAR that it is accompanied by in the dataset was calculated from the meteorological data.

Furthermore, with three factors from the sensitivity test that explained a large part of the variation in the TMCF GPP (T_{air}, PAR and LAI), additional simulations were performed to estimate TMCF GPP under tropical lowland forests conditions. The maximum values for the drivers T_{air} and PAR and structural parameter LAI did not originate from one particular tropical lowland site, but were set to values within the

range of values known from the literature (*e.g.* Carswell *et al.* 2002, Domingues *et al.* 2005, Fisher *et al.* 2007, Mahli *et al.* 2009). First, T_{air}, PAR and LAI were increased individually, leaving all other factors as observed in the TMCF. Additionally, GPP was simulated with a combination of two from the three, or all three set to the 'tropical lowland' value (Table 5). As in the previously described simulations, daily and seasonal variations were kept proportional to the original observed values and short wave radiation was changed concordantly with PAR.

5.3 Results

5.3.1 Meteorology

T_{air} did not show strong seasonal differences throughout the year (Figure 1a) and the mean average daily T_{air} was 10.45 ºC ± SD 0.56. The warmest month was in the wettest period in November 2008 (11.22ºC ± SD 1.13), while the coldest period was in the 'dry' season in June 2009 (9.88 ºC ± SD 1.04). The range of average daily VPD values was not very distinct (Figure 1c). Maximum daily VPD did not exceed 1.305 kPa, while average daily VPD differed throughout the year from 0.52 kPa to 0.04 kPa. Throughout the year, the range of average daily VPD was higher throughout the drier months May-September, but even throughout the dry season there are days with low average VPD values (< 0.007). The month with the highest mean average daily VPD was August 2008 (0.214 kPa ± SD 0.123) and the month with the lowest mean average daily VPD was December 2009 (0.077 kPa ± SD 0.059). Average daily PAR was most variable throughout August 2008 - December 2008. April 2009 had the lowest mean average PAR (199 μmol m^{-2} s^{-1}± SD 87) and September 2008 the highest (289.49 μmol m^{-2} s^{-1} ± SD 118.9) The maximum observed PAR value was 2458 μmol m^{-2} s^{-1}, the average daily maximum was 1308.8 μmol m^{-2} s^{-1} ± SD 484.8. Rainfall for the simulated year was 1724 mm; a 'wet season' can be distinguished from October 2008 until March 2009 (Figure 1d), with 46% of the rainfall occurring from December 2008 until the end of February 2009). Finally, the average LAI throughout the year ranged from 4.0-4.38, while the spatial variability in the 1 ha plot was much higher (Table 3).

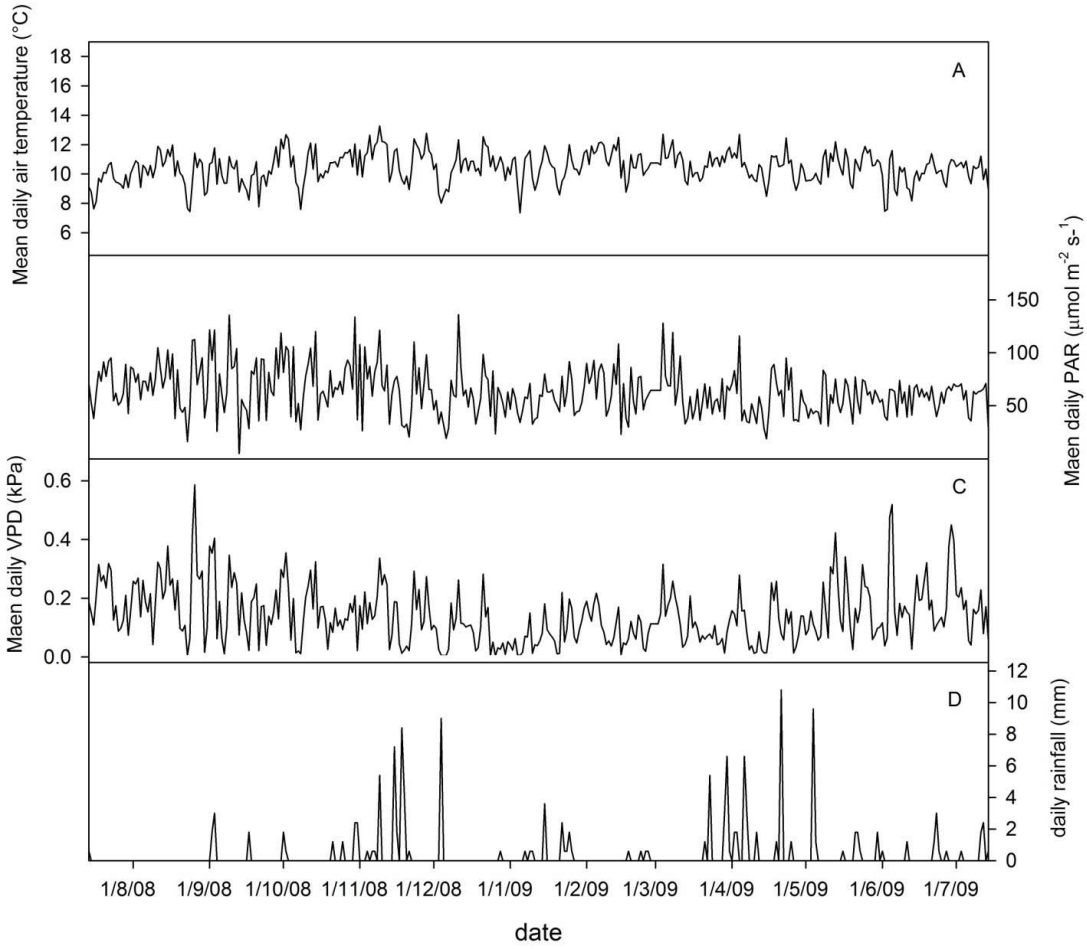

Figure 1. Meteorological variables as measured at the research site and used for driving the standard run of the SPA model for the period between 14 July January 2008 and 13 July 2009 December. a) mean daily temperature, b) mean daily photosynthetically active radiation (PAR), c) mean daily vapour pressure deficit (VPD), d) and total daily rainfall.

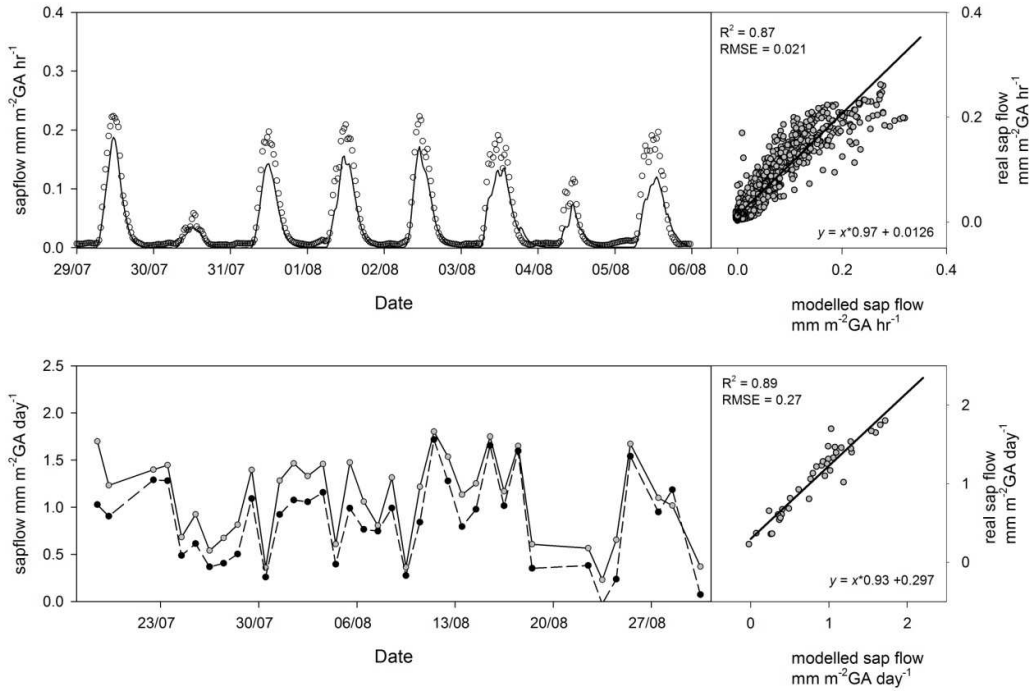

Figure 2. Simulated and observed stand-scale hourly and daily sap flow from for the period from 18 July 2008 to 31 August 2008 per m^2 ground area (GA). In the upper panel modelled sap flow is represented by lines, in the bottom panel with black circles. The modelled sap flow represents the result from the SPA run with standard inputs (Table 3) and the observed values were scaled up to stand scale from 12 trees growing in the simulated research plot and then averaged per m^2.

5.3.2 Modelled sap flux

The model simulates the hourly sap flow accurately, both for the values of simulated sap flow and for the hourly pattern during the daytime (Figure 2). R^2 for the hourly modelled sap flow compared with modelled data was 0.87, with a root mean square error (RMSE) of 0.022 mm m^{-2} GA hr^{-1}, while R^2 for daily sap flow was 0.89, with an RMSE of 0.27 mm m^{-2} GA day^{-1}. For the last days of the collected sap flow data, the model overestimates the sap flow, but no specific factor explaining this discrepancy could be found. The simulated SWC fitted less well with the observed field data (R^2 = 0.61) though the annual simulated pattern followed the field data (RMSE = 0.0516, slope = 1.05, intercept = 0.0078).

5.3.3 Modelled GPP

The simulated annual GPP was 16.24 t C ha^{-1} yr^{-1}. Daily GPP varied from 19.14 kg C ha^{-1} day^{-1} – 68.65 kg C ha^{-1} day^{-1}, with a mean value of 44.92 ± SE 0.42kg C ha^{-1} day^{-1}. Total GPP varied throughout the year (Figure 3a), but without any distinct seasonal pattern. Over the whole year, the average daily GPP for the total canopy derived from direct radiation is not significantly different from GPP derived from indirect radiation (P = 0.08), though there are distinct periods where the GPP derived from indirect radiation is higher than GPP derived from direct radiation and *vice versa* (Figure 3b). For a 70 day period between 8 December 2008 and 15 February 2009, a paired t-test showed that the contribution to GPP by photosynthesis from diffuse light is higher than from direct light (P<0.001), while in the 70 day period from 1 May 2009 to 9 July 2009 the contribution the total canopy GPP by photosynthesis from direct light is higher than from diffuse light (P<0.001).

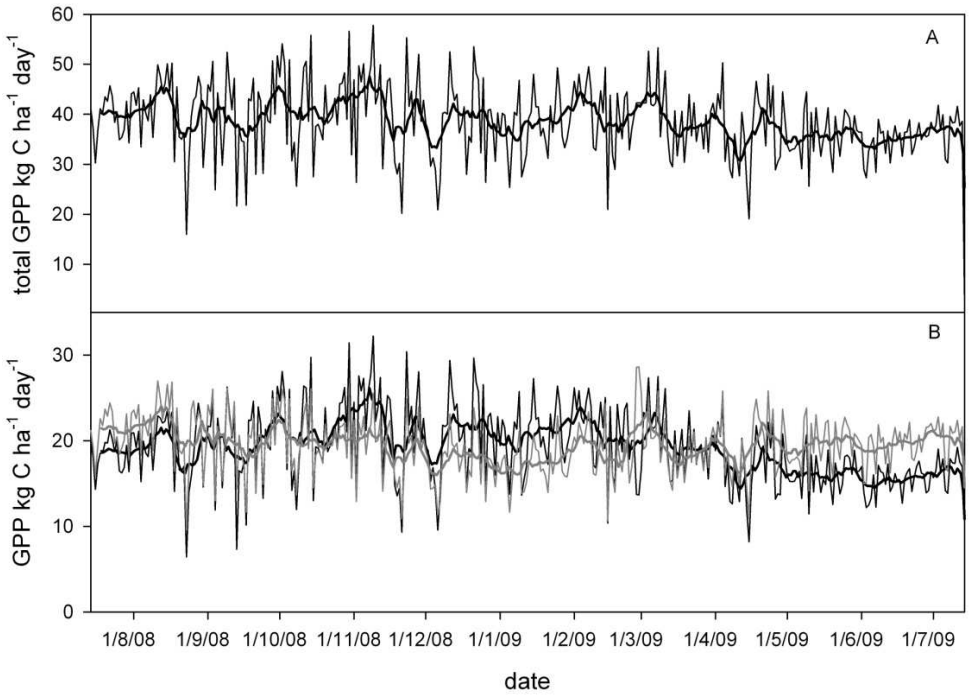

Figure 3. Simulated daily GPP on a stand scale, resulting from the standard input in the SPA model from 14 July 2008 to 13 July 2009. Panel a shows the total daily simulated GPP, while in panel b simulated daily GPP derived from direct radiation (black line) and total indirect radiation (grey line) are shown. Thicker lines represent 10-day averages.

5.3.4 Sensitivity analyses

The most important controls on the simulated GPP are shown in Figure 4, and all structural parameters and meteorological drivers included in the sensitivity analysis are listed in Table 3 in order of importance. Simulated GPP was most sensitive to changes in V_{cmax} and J_{max} (Figure 4a). As for meteorological drivers, the most important factor was irradiance (both PAR and total shortwave radiation, Figure 4c). Other important factors included T_{air}, and LAI (figure 4b and 4e). The sensitivity to changes in LAI depended on whether N levels were kept constant at a foliar or at a canopy level. With canopy N kept constant (and hence changing on a foliar basis), a change from the average LAI of 4.17 to the minimum of 2.6 resulted in a decrease in GPP of 3.2%, while changing LAI to the maximum of 5.6 increased GPP with 0.1%. However, with foliar N kept constant (and hence changing at canopy level), the reduction in GPP with the minimum LAI was 29.3% and the increase in GPP with the maximum LAI resulted in a 19.0% increase in yearly GPP (Figure 4e). This indicates the amount N in the canopy (which in the model corresponds to V_{cmax} and J_{max}) is more important than an increase in leaf area itself. GPP was not sensitive to the range of observed mean daily VPD (Figure 4d).

The diffuse fraction of light is also an important factor controlling GPP (Table 3, Figure 4F). However, generally, higher fractions of diffuse radiation often imply lower levels of total PAR and *vice versa*, with consequences for GPP (Figure 5). Therefore, in practice, an increase in total PAR will coincide with lower levels of diffuse radiation, resulting in lower GPP levels if the fraction of diffuse radiation would stay the same. Similarly, low levels of total PAR can still increase GPP if a higher portion of the radiation is diffuse.

No effects on simulated GPP were found when changing capacitance or ι. Furthermore, changing the root biomass or in the aboveground or belowground resistivity between the maximum and minimum values listed in Table 3 did not alter the value of simulated GPP. For a 1% reduction in GPP, plant hydraulic conductance needed to be reduced to 0.2 mmol m^{-1} s^{-1} MPa^{-1}, which is a reduction of more than 90% from the minimum plant conductivity observed in the field (Table 3). Likewise,

root resistivity needed to be 33.5 times as high as the standard value in order to establish a decrease in TMCF GPP of 1%. Furthermore, GPP showed a steep decline in GPP when decreasing SWC to 0.1, though not within the ranges of the observed maximum and minimum SWC (0.196-0.367 m^3 m^{-3}). This implies that within the range of observed soil water conditions and hydraulic structural parameters, GPP for the simulated TMCF is very insensitive to changes. Finally, GPP also showed little sensitivity to changes in VPD (Figure 4d). For example, increasing the daily average VPD by 150 % resulted in a GPP reduction of 4.3 %.

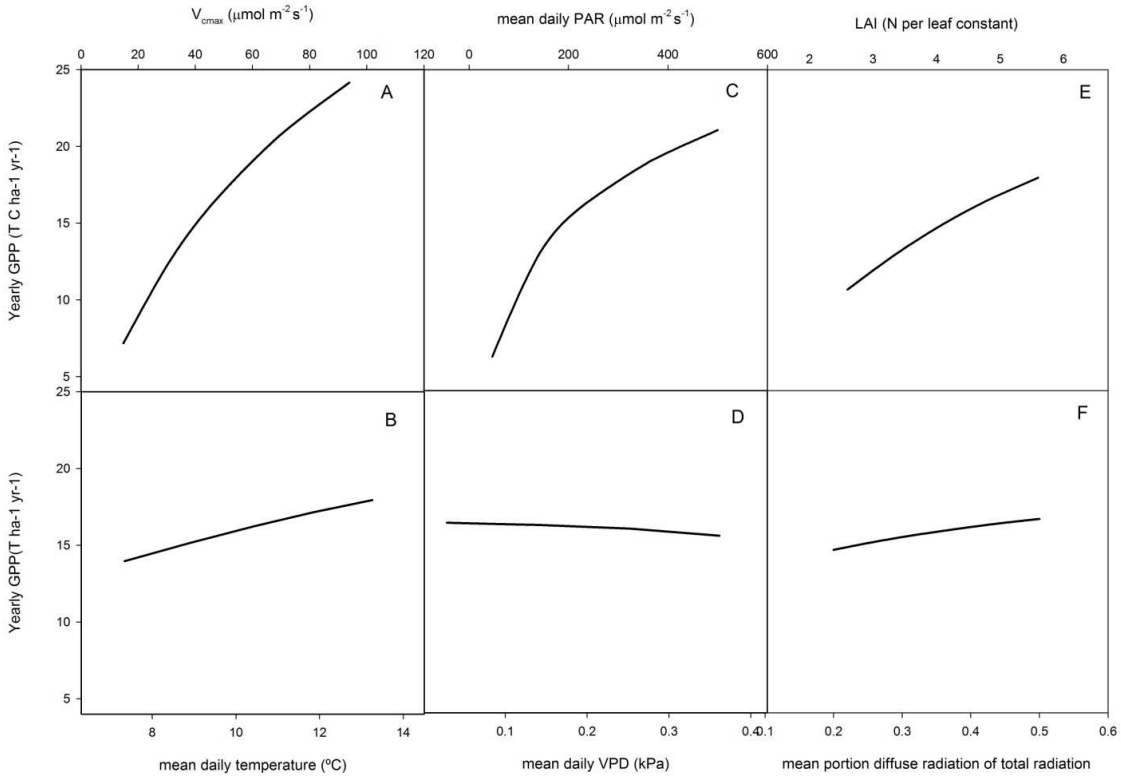

Figure 4. Results from the one-dimensional sensitivity analyses for six important factors controlling annual TMCF GPP. Note that for panel A, both V_{cmax} and J_{max} were changed concurrently, while only V_{cmax} is listed on the X-axis.

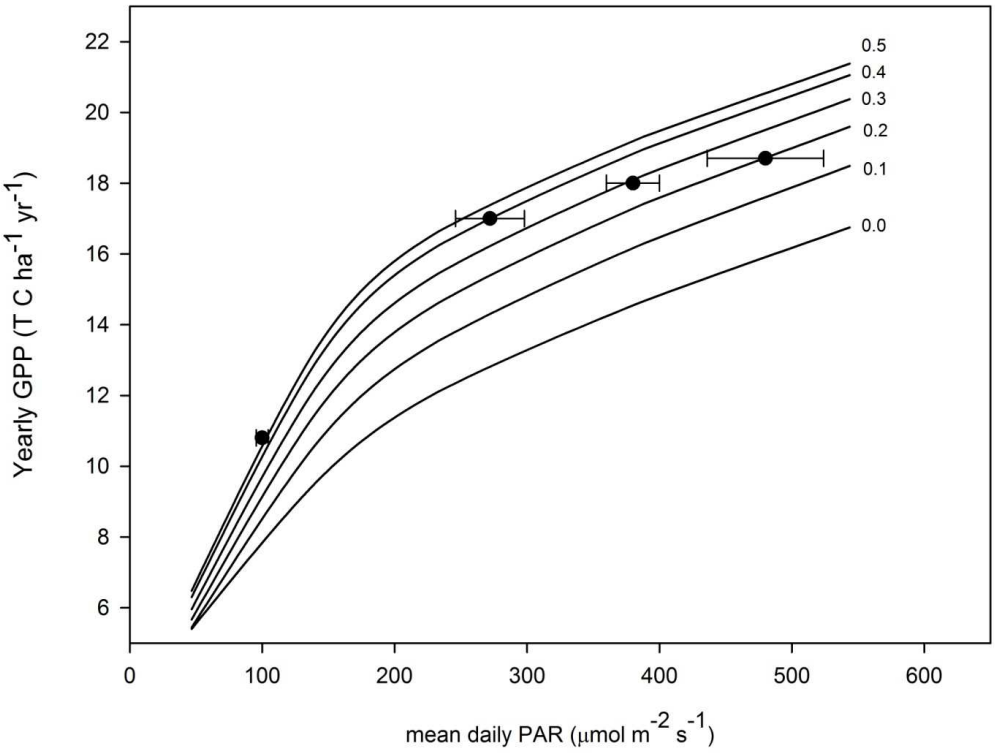

Figure 5. One dimensional sensitivity analyses of mean daily photosynthetic active radiation (PAR) on TMCF GPP for different mean fractions of diffuse ration (0.0-0.5). Average daily shortwave radiation changed accordingly to PAR with each analysis. Black circles represent the observed mean daily PAR ± SE at the corresponding observed fraction of diffuse radiation (n = 8-26).

5.3.5 Increasing T_{air}, PAR and LAI

When increasing T_{air}, PAR and LAI to tropical lowland values individually, both T_{air} increased the TMCF GPP around 30%, while changing LAI and daily average PAR increased GPP less profoundly, but still with 17% and 15% respectively (Table 4). Increasing all three factors simultaneously, increased GPP up to 28.3 t C ha^{-1} yr^{-1}, which was an increase of more than 75%.

Table 4. The factors used to simulate TMCF under conditions similar to lowlands tropical rainforests, together with their units and maximum values, as well as the simulated GPP when these factors were changed alone or together, and the % increase of GPP compared with the standard TMCF conditions (GPP = 16.24 t C ha^{-1} yr^{-1}).

Factor	Unit	Value	Simulated GPP (t C ha^{-1} yr^{-1})	% Increase in GPP
T_{air}	°C	26	21.2	30.3
PAR	μmol m^{-2} s^{-1}	280	18.6	14.9
LAI	-	5.5	19.0	17.2
T_{air} + PAR			24.1	49.4
T_{air} + LAI			24.6	52.3
PAR + LAI			21.7	34.5
T_{air} + PAR + LAI			28.3	75.4

5.4 Discussion

5.4.1 Simulating GPP

The modelled SWC and sap fluxes were consistent with the seasonal observed pattern of SWC, and more importantly with observed hourly and daily sap flow patterns. The consistency with observed hourly sap flow patterns, which was observed as well by earlier studies using the SPA model (Fisher *et al.* 2006, 2008), implies that the SPA model simulates diurnal stomatal activity accurately. This supports the use of the SPA model to simulate C-uptake, even though a direct comparison with an observed canopy CO_2 flux data set was not possible. Furthermore, the modelled results are consistent with an independent calculation made by Malhi *et al.* (2009), where GPP was estimated by summing all ecosystem respiration and growth components and assuming approximate steady state. SPA has an error of about 10% (Fox *et al.* 2009), thus directly modelled annual GPP was 16.24 ± 1.6 t C ha^{-1} yr^{-1} in this study, which is slightly higher, but not very different from the indirect estimate by Malhi *et al.* (2009) of 14.9 ± 1.9 t C ha^{-1} yr^{-1}.

GPP at the simulated TMCF site was about half the values reported from lowland tropical rainforests, which vary between 30 - 36 t C ha^{-1} yr^{-1} for neotropical rainforests in Brazil (*e.g.* Malhi *et al.* 1998, Carswell *et al.* 2002, Chambers *et al.* 2004, Hutyra *et al.* 2008) and 31-32 t C ha^{-1} yr^{-1} in Asia (Hirata *et al.* 2008, Kosugi *et al.* 2008). This is consistent with the general paradigm (based on NPP measurements) that GPP in tropical montane cloud forests (TMCF) is lower than that of lowland tropical rain forests (*e.g.* Bruijnzeel and Veneklaas 1998, Waide *et al.* 1998, Moser *et al.* 2007). However, the ratio between lowland rainforest and TMCF NPP might not hold for GPP. Girardin *et al.* (in press) observed a $NPP_{lowland}$: NPP_{TMCF} ratio between 2.7 and 3.3. Their study included the same TMCF research site that was simulated here, as well as two different lowland tropical rainforest in south east Peru. Though we do not have SPA-based estimates of GPP from the same lowland rainforest sites, with the previously reported values for neotropical GPP (30 -36 t C ha^{-1} yr^{-1}), the $GPP_{lowland}$: GPP_{TMCF} would range from 1.8 to 2.2. This implies that per unit of C-uptake (GPP), the lowland rainforests can invest a larger part of their C-uptake in net productivity than a TMCF. Or in other words, the TMCF autotrophic respiration is proportionally larger to its GPP than in lowland forests. Of course, more TMCF GPP estimates or measurements are needed to determine if the $GPP_{lowland}$: GPP_{TMCF} ratio we observed here is valid for other TMCFs as well. However, hardly any TMCF GPP estimates are available. The only other study to our knowledge to have predicted TMCF GPP through a modelling exercise is the simulation of GPP in the Luquillo Mountains, Puerto Rico by Wang *et al.* (2003). They predicted annual GPP values of 60.32 – 24.08 t C ha^{-1} yr^{-1} in tropical forests over an altitudinal range of 450-1050 m a.s.l. respectively, using a canopy process model driven with simulated climate data from a topographical climate model and remotely sensed LAI data derived from NDVI measurements. Compared to their estimate, the GPP value of 16.24 ± 1.6 t C ha^{-1} yr^{-1} from this study is rather low. However, Wang *et al.* (2003) overestimated GPP up to 43% compared with field observations. Furthermore, compared to our study, the higher GPP levels simulated in the TMCF of Wang *et al.* (2003) can also be explained by the higher average T_{air} and LAI values in their simulated forests. Therefore, though Wang *et al.* (2003)

found higher TMCF GPP rates, we regard it unlikely that the SPA model underestimated the GPP of our study site.

5.4.2 Controls on GPP

From the sensitivity analyses, the most important meteorological controls on TMCF GPP were T_{air} and PAR, while for the structural parameters, the photosynthetic capacity per leaf area (V_{cmax} and J_{max}) and LAI with a constant leaf N were key controls. Below, the importance of these factors are discussed separately.

Temperature

Low daily temperatures have for a long time been suggested to limit TMCF growth, both as a direct kinetic effect on photosynthesis, and as a negative control on nutrient mineralization rates (Bruijnzeel and Veneklaas 1998, Waide et al, 1998). The latter effect is not represented in SPA, but the results presented here confirm the temperature sensitivity of TMCF photosynthesis to T_{air}. Within the range of observed TMCF values, T_{air} was not the most important factor determining GPP (Figure 4), but when increased to tropical lowland values, GPP increased with more than 30 % (Table 4), indicating the difference in mean T_{air} between TMCFs and lowland forests explains for a substantial part the difference in TMCF and lowland forest GPP. It has to be kept in mind that in the analysis, other meteorological drivers were held at TMCF level. *In situ* changes of T_{air} are most likely accompanied by increases in VPD, and increases in VPD are known to cause midday stomatal closure in tropical lowland forests, which limits C-uptake (*e.g.* Brodribb and Holbrook 2004). Therefore, if the modelled TMCF would experience lowland rainforest T_{air} values, the accompanying higher VPD would probably cause a similar stomatal closure, with a lower than 30% increase of GPP as a consequence. Furthermore, although temperature increases are observed and predicted in the tropical Andes (Vuille *et al.* 2003, Urrutia and Vuille 2009), plants can adapt their photosynthetic rates to average growing temperatures (Berry and Bjorkman 1980, Atkin *et al.* 2006, Way and Sage 2008, but also see Ow *et al.* 2008). No data is available on the long-term temperature sensitivity of TMCF photosynthesis, but if long term increases of T_{air} in practice can

result in changes in the photosynthetic capacity, or their temperature sensitivities, the modelled results presented here cannot simply be extrapolated for future climate scenarios. Nevertheless, the results from the GPP simulations emphasize the importance of T_{air} in explaining the difference in TMCFs and lowland rainforests productivity.

Light conditions

The typical cloudy conditions of TMCFs cause them to have lower mean PAR levels compared with other tropical ecosystems (Letts and Mulligan 2005). Therefore, incident radiation is assumed to be a strong control on TMCF (*e.g.* Bruijnzeel and Veneklaas 1998, Letts and Mulligan 2005). Indeed, when PAR levels were increased to tropical lowland averages, simulated GPP increased by almost 30 %, showing that like T_{air}, PAR is an important factor in explaining the difference between TMCF and tropical lowland GPP. However, an increase in daily average PAR as a consequence of less cloud cover will be accompanied by a reduction in the fraction of diffuse radiation. Changing mean incident PAR, as well as the fraction of PAR that is diffuse showed that when combining an increase in PAR with the corresponding decrease in fraction of diffuse radiation, GPP still increased. However, the increase was not as large as when the diffuse fraction would have stayed the same. For example, based on the meteorological dataset used for this study, increasing the daily mean irradiance from 270 to 470 μmol m^{-2} s^{-1} reduced the amount of received fraction diffuse radiation from 0.4 to 0.2. As a result, GPP increases with 10%, rather than 18% (Figure 5). The opposite holds true as well though, reductions in GPP as a result of lower PAR levels are partially offset by increases in GPP due to larger fractions of diffuse radiation. Overall, the modelled results underline the importance of PAR, as well as diffuse light conditions in determining TMCF GPP. To fully understand the effects of diffuse radiation and PAR quantity on TMCF productivity, field studies measuring GPP *in situ* and diffuse and direct radiation in the TMCF canopy are urgently needed.

Photosynthetic parameters and nitrogen

Tanner *et al.* (1998) hypothesized that TMCF productivity is limited by available N. Indeed, the sensitivity analyses showed that GPP was very sensitive to changes of the photosynthetic parameters V_{cmax} and J_{max} (Figure 4a), which are two parameters that in general increase with foliar N content, an assumption that is embedded in the SPA model as well. However, when averaged to the same standard temperature (*e.g.* 25 °C or 30 °C), there is little difference between and tropical lowland forests and TMCFs in N or V_{cmax} and J_{max} when expressed per leaf area. (*e.g.* Carswell *et al.* 2000, Domingues *et al.* 2007, Meir *et al.* 2007). Therefore, it is unlikely that differences in photosynthetic capacity itself explain the difference in TMCF GPP and tropical lowland GPP. More likely, the temperature sensitivity of both V_{cmax} and J_{max} cause the *in situ* C-uptake per leaf area to be lower under TMCF temperatures. In addition, the strong sensitivity of GPP to changes in V_{cmax} and J_{max} provide indirect support for the hypotheses that TMCF productivity is limited by N, on the premise that a higher N availability would be invested in the photosynthetic apparatus. However, fertilisation experiments have shown that N addition in TMCFs do not always lead to increases in foliar N concentrations (Tanner *et al.* 1992, J. Fisher, pers. comm.). Therefore, without a uniform foliar response to N addition in TMCFs in experiments, it would stretch too far to conclude from the modelled results that increases in available N inevitably lead to higher GPP rates.

Leaf area index

The LAI in the SPA model can be varied with either the foliar N kept constant, or with the total canopy N kept constant (Table 3). The simulated GPP was practically insensitive to choosing the latter combination, showing that the sensitivity of GPP to LAI changes depends on what happens with the foliar N content. If the foliar N stays constant with increasing LAI, it effectively means that there is more N in the simulated canopy, and hence more photosynthetic capacity per ground area. Lower TMCF LAI values in TMCFs have been hypothesised to be an important limitation in TMCF GPP (Bruijnzeel and Veneklaas, 1998), and the results from our GPP simulations confirm this notion. However, as Bruijnzeel and Veneklaas (1998) pointed out, increases in LAI can also increase the amount of foliar biomass that

respires, providing the LMA would stay constant. An increase in TMCF GPP as a result of increased LAI, a relationship that was non-linear (Figure 3), does therefore not necessarily correspond to an increase in ANNP, as this will depend on the ratio of C-uptake and autotrophic respiration. In addition, the strong LAI-GPP relationship provides more indirect support for the idea that TMCF productivity is limited by N (Tanner et al, 1998). However, just as fertilization experiments showed no uniform response in photosynthetic parameters, fertilization with N does not always increase TMCF LAI (Harrington *et al.* 2001). Therefore, although the SPA simulations show that a higher LAI with a similar photosynthetic capacity increases TMCF GPP, it is unclear how plausible a scenario of increasing LAI under increased N availability is in practice.

Hydraulic controls

GPP was remarkably insensitive to changes in the belowground root resistivity and root biomass, as well as aboveground hydraulic conductivity, capacitance and ι (Table 3). This insensitivity is probably explained by the relatively high SWC and the low evaporative demand from the atmosphere throughout the year. Apparently, no or little hydraulic stress is imposed on the TMCF hydraulic functioning within the boundaries of the observed SWC and VPD, so changes in the conductance of the hydraulic pathway below- or aboveground have no consequences for stomatal opening, and consequently not for C-uptake. These results agree with the observations that TMCF trees rarely, if at all, experience severe soil water deficits (*e.g.* Kapos and Tanner 1985, Bruijnzeel *et al.* 1993, Motzer *et al.* 2005), contrary to lowland rainforests (*e.g.* Fisher *et al.* 2008, Brodribb and Holbrook 2004) and confirm the hypothesis from Bruijnzeel and Veneklaas (1998) that periodic water shortage is an unlikely explanation for low TMCF productivity. A separate sensitivity analysis showed that GPP decreased only if SWC values lower than the observed SWC minimum were used in SPA. Therefore, predicted reductions in precipitation in the Eastern tropical Andes as a consequence of climate change (Vuille *et al.* 2003, Urrutia and Vuille 2009), could reduce annual TMCF C-uptake in this region. However, under current climatic conditions no evidence for drought stress is observed.

5.5 Conclusions

We presented the first simulated annual GPP of a TMCF based on direct field-measurement of biochemical photosynthetic capacity (V_{cmax} and J_{max}). The model (SPA; Williams *et al.* 1996, Fisher *et al.* 2007) accurately predicted water use by the TMCF when comparing observed and modelled sap flow rates. Furthermore, the directly modelled estimate of GPP derived in this study was similar to an independent estimate for the same forest made by summing respiration and growth terms. TMCF GPP cannot be measured in a direct manner, but the accurate simulation of the TMCF sap flux and the relatively close agreement between GPP estimation methods provides confidence in our estimate of GPP of 16.24 ±1.6 t C ha^{-1} yr^{-1}. This value is about half the GPP reported from neotropical rainforest (30 -36 t C ha^{-1} yr^{-1}), making the $GPP_{lowland}$: GPP_{TMCF} ratio (1.8-2.2) lower than observed $NPP_{lowland}$: NPP_{TMCF} ratios (2.7-3.3), suggesting relatively higher autotrophic respiration rates for TMCFs. Overall, the three key factors controlling TMCF GPP were T_{air} , PAR, and LAI. Increasing these three factors to tropical lowland values increased simulated GPP up to 75%, showing that the difference in T_{air}, PAR and LAI between TMCFs and tropical lowland forests is important in explaining the difference in productivity between these different tropical forests. Additionally, our analysis shows that higher PAR levels will only increase GPP if the diffuse fraction of radiation does not decline too much. Therefore, the cloudy condition of TMCFs should not be simply regarded as a negative control on TMCF GPP by reducing the total irradiance, since cloud presence increases the fraction of diffuse radiation, hence limiting the decrease in GPP. Higher levels of N, either through higher photosynthetic capacity per leaf area or through higher LAI values, increased simulated GPP. This provides indirect support for the hypothesis that TMCFs are N limited, even though in practice fertilisation experiments have shown idiosyncratic results. Finally, hydraulic stress is not an important factor controlling TMCF GPP under the range of field observations, but could become a more important control under a substantially drier climate.

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Chapter 6: General discussion

The previous four chapters have presented and discussed different factors that potentially control the carbon (C) gain in a tropical montane cloud forest (TMCF) in Peru, either on a leaf level (Chapter 2 and 3), canopy level (Chapter 3 and 5) or by investigating the role of different environmental variables on gross C-uptake (Chapter 4 and 5). In this chapter, the results of Chapter 2-5 are synthesized and reviewed in order to answer the main underlying question of this thesis: What controls C-uptake in a TMCF? Suggestions for further research are given throughout the discussion.

6.1 Productivity in TMCFs

As mentioned in the introduction of this thesis, the low (aboveground) productivity of TMCFs has been the subject of studies for several decades (*e.g.* Grubb 1977, Vitousek 1984, Tanner *et al.* 1998, Vitousek 1998, Kitayama and Aiba 2002). Especially the aboveground and C stocks and related processes have received attention, for example through stem increment studies, or comparisons of leaf area index (LAI) between TMCFs (Grubb and Whitmore 1966, Proctor *et al.* 1989, Veneklaas 1991, Tanner *et al.* 1992, Schuur and Matson 2001, Kitayama and Aiba 2002, Leuschner *et al.* 2007, Moser *et al.* 2007), while the interest in belowground processes regarding the TMCF C-cycle are more recent (*e.g.* Girardin *et al.* in press, Iost *et al.* 2008, Zimmermann *et al.* 2009a, Zimmermann *et al.* 2009b). Overall, the general patterns regarding above and belowground allocation of C show that TMCFs allocate relatively more C belowground compared with tropical lowland forests, but that both the aboveground net primary productivity (ANPP) and belowground net primary productivity (BNPP) are lower (Girardin *et al.*in press, Roderstein *et al.* 2005, Leuschner *et al.* 2007, Zimmermann *et al.* 2009b). However, these results on net productivity do not necessarily inform us about the value of the gross primary productivity (GPP) or the factors controlling gross C-uptake of a TMCF.

Photosynthesis, the process that underpins gross C gain, does not differ much on a leaf area basis between TMCFs and lowland tropical rainforests (Hikosaka *et al.* 2002, Letts and Mulligan 2005). However, little information is available on what variables control TMCF C-uptake. Overall, the lower temperatures, lower intensities of radiation as a consequence of frequent cloud events and low nitrogen (N) availability have been put forward as the main factors limiting TMCF productivity (Grubb 1977, Bruijnzeel and Veneklaas 1998, Tanner *et al.* 1998, Vitousek 1998, Letts and Mulligan 2005, Raich *et al.* 2006). Other hypotheses, though they are considered to be of less importance, include periodic water shortages because of shallow soils, water logging of the soils because of low atmospheric evaporative demand, and high phenolic concentrations of the TMCF litter that reduce decomposition and hence nutrient availability (Bruijnzeel and Veneklaas 1998). Not all of the hypotheses listed here have been tested explicitly in this thesis. Nevertheless, they are discussed below in the context of the conducted research.

6.2 Temperature

The lower temperatures in TMCFs have for a long time been thought to limit TMCF productivity (Grubb 1977, Weaver *et al.* 1986, Bruijnzeel and Veneklaas 1998). Chapter 3 showed that the maximum carboxylation efficiency of the Rubisco enzyme (V_{cmax}) and the maximum electron transport rates (J_{max}) in the research site at 2990 m a.s.l. were similar to values found in tropical lowland forests, when expressed on a leaf area basis and when standardised for the same leaf temperature. However, when standardised to the research sites' mean annual temperature of 12.5 °C, both V_{cmax} and J_{max} decrease considerably. As a consequence, under ambient conditions, the photosynthetic capacity of TMCF leaves is much lower than in tropical rainforests where the ambient temperatures are higher (~26 °C). In addition, Chapter 5 showed that simulated GPP was sensitive to changes in the average daily temperature, and when increased to 26°C, simulated TMCF GPP increased more than 30%. Although an increase to such high mean temperatures in a TMCF would probably be accompanied by factors that limit productivity (*e.g.* higher VPD values), this result underlines the importance of temperature as a direct control in TMCF productivity.

On a longer time scale, however, the temperature sensitivity of foliar CO_2 exchange for TMCF vegetation has remained unexplored so far. When plants are allowed to acclimate to a wide range of temperatures, they can adjust their photosynthesis levels to an extent where no significant differences in photosynthesis between growth temperatures can be observed (Atkin *et al.* 2006; Campbell *et al.* 2007). Therefore, caution should be taken not to extrapolate the results from the SPA model to anticipated increases in temperature under climate change. Therefore, to gain more insight on the effects of temperature changes on TMCF gross productivity, more (long term) studies on photosynthesis and foliar respiration and their sensitivity to *in situ* temperature changes are needed.

Next to a direct effect of temperature on foliar metabolism and C-uptake, there are several possible pathways by which lower temperatures at higher altitudes can result in impaired nutrient supply or uptake. Firstly, lower temperatures can result in a reduction of decomposition rates (*e.g.* Wilcke *et al.* 2002, Iost *et al.* 2008), or in lower membrane transporter activity and thus lowered nutrient uptake rate by roots (Bassirirad *et al.* 2001). A higher nutrient availability as a consequence of temperature increases can result in a positive feedback, where higher nutrient availability will improve the litter quality, which in turn will stimulate nutrient cycling (*e.g.* Bardgett *et al.* 1998). However, in many models, like the SPA model used in Chapter 5, these aboveground-belowground interactions are not represented (Schröter *et al.* 2004). Therefore, no conclusions regarding these temperature-productivity interactions can be drawn from the results presented in this thesis. Overall, the lower temperatures of TMCFs compared with lowland rainforest are an important factor in determining the TMCF productivity both directly to the kinetics of photosynthetic parameters, and probably as well through controlling mineralisation rates.

6.3 Leaf traits and nutrient limitations on TMCF productivity

Foliar N and phosphorus (P) content, together with leaf mass per area (LMA) and leaf longevity are known to scale with photosynthetic capacity and foliar respiration

rates on a global scale. This is often described as the 'global leaf economic spectrum', which is schematically represented in Figure 1 (Wright *et al.* 2004). The relationship observed between N and LMA in the species of the Kosñipata valley, discussed in Chapter 2, show that the TMCF species fall within the ranges of this spectrum. Furthermore, the N:P ratios throughout the altitudinal transect suggest that, except for the site at 2990 m a.s.l., the TMCF species throughout the Kosñipata valley are P, rather than N limited. This contradicts the general theory put forward by Tanner *et al.* (1998), that TMCFs are N limited.

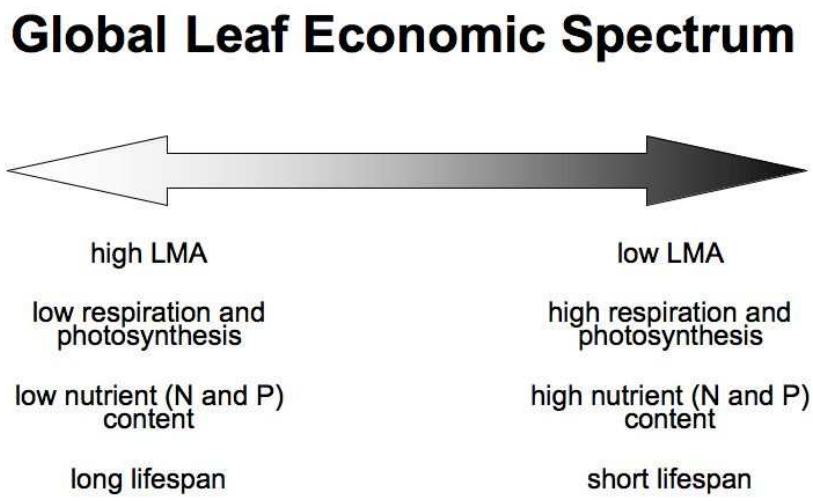

Figure 1. A schematic overview of the 'global leaf economic spectrum', based on Wright *et al.* (2004).

At the site at 2990 m a.s.l., the only site where foliar N:P ratios were indicating N limitation, the data presented in Chapter 3 showed a significant, though noisy, relationship between N and the maximum carboxylation efficiency of the Rubisco enzyme (V_{cmax}). More importantly, both V_{cmax} and the maximum electron transport rates (J_{max}) were similar to values found in tropical lowland forests, when expressed on a leaf area basis and when standardised for the same leaf temperature. Based on these observations, the TMCF leaves are not more N limited than leaves from

tropical lowland forests. However, as mentioned above, when standardised to the mean annual research site temperature of 12.5 °C both V_{cmax} and J_{max} decrease considerably. In addition, the results from Chapter 3 showed that TMCF V_{cmax} is underestimated by using V_{cmax}-N relationships reported from tropical lowland forest. The sensitivity analysis of the modelled GPP in Chapter 5 showed a large sensitivity of TMCF GPP to changes in photosynthetic capacity, emphasizing the importance of quantifying V_{cmax} and J_{max} accurately. Given these two latter observations, TMCF C-uptake should not be modelled by using nutrient-photosynthesis relationships known from other biomes and for their N use efficiency (NUE), TMCF species should be regarded as a separate plant functional type.

As pointed out by Tanner *et al.* (1998), true nutrient limitation to productivity can only be investigated experimentally with a nutrient addition experiment. N (and P) addition in TMCFs have resulted in a variety of responses in other studies. Some studies showed an increase in stem growth and increases in leaf area index (LAI) after fertilisation with these nutrients (Tanner *et al.* 1998, Tanner *et al.* 1992). The sensitivity analyses of the modelled GPP values in Chapter 5 also showed that increasing canopy N through higher photosynthetic capacities, as well as increasing canopy N through higher LAI values both, increased TMCF GPP. Therefore, if higher N availability would increase GPP of the studied site, either through more N-deposition (Fabian *et al.* 2005, Boy *et al.* 2008), or higher mineralization rates caused by predicted warming (Vuille *et al.* 2003, Urrutia and Vuille 2009), this will most probably be mediated through either increases in LAI, or through increases in photosynthetic capacity per leaf area. However, with experimental data on N addition lacking in this thesis, this remains to be tested with more long term field experiments. Overall, based on the results presented in this thesis, N limitation might not play an important role to gross C-uptake for most of the vegetation types found throughout the Kosñipata valley, since the observed N:P ratios do not indicate stoichiometrical constraints by foliar N concentration. However, the results of the modelling exercise indicate that increases of canopy N at the site as 2990 m a.s.l. have potential to increase the productivity there.

6.4 Radiation

Lower levels of photosynthetically active radiation (PAR) have been put forward as an important control on TMCF gross C-uptake, since the frequent presence of clouds reduces the total amount of radiation received by the TMCF canopy up to 50% compared with tropical forests at lower altitudes (Bruijnzeel *et al.* 1993, Letts and Mulligan 2005). However, the role of diffuse radiation as a consequence of the cloudiness has remained hardly investigated for TMCFs, even though the importance of diffuse radiation for canopy C-uptake has come to attention over the last decade (*e.g.* Roderick *et al.* 2001, Graham *et al.* 2003, Alton *et al.* 2007, Knohl and Baldocchi 2008, Mercado *et al.* 2009). The results of the sensitivity analyses of modelled GPP at the site at 2990 m a.s.l. altitude showed that both the amount of received PAR influences the GPP in this TMCF, as well as its diffuse fraction (Chapter 5). When increased to tropical lowland values, PAR was an important factor in explaining the difference in TMCF and tropical lowland GPP. Furthermore, the results showed that the fraction of diffuse radiation is important in controlling TMCF GPP, because in reality, an increase in mean daily PAR will coincide with a decrease in diffuse fraction, and *vice versa*. Therefore, a reduction GPP through decreases in incident PAR as a consequence of cloudiness is offset partially by the positive effect on canopy C-uptake from the coinciding increase in diffuse radiation. The cloudy conditions in TMCFs should therefore not be regarded as just a negative control on TMCF GPP.

It is important, however, to realize that modelled results of GPP are estimates, and not direct measurements. To fully understand the influence of the TMCF cloudiness on received radiation throughout the canopy and consequently on C-uptake, observations of total PAR and the diffuse fraction throughout the canopy are needed. Especially since the scattering and attenuation of radiation throughout the canopy will be different if the canopy itself is covered in mist (Monteith and Unsworth 1990), something not incorporated in the SPA model used in Chapter 5. Additionally, a recent study on sunflowers showed that diffuse and direct radiation also have different consequences for photosynthesis on a leaf level (Brodersen *et al.* 2008), although this is a research area that is not explored in field conditions yet. In

sum, to get a better understanding of the radiation regime within the cloud forest canopy, measurements of diffuse and direct radiation throughout the canopy are needed, and studies on the importance of diffuse PAR on leaf level C-uptake would be the next logical step in TMCF C-uptake research.

6.5 Other environmental controls

Other suggested controls on TMCF productivity have mostly been related to the (soil)water status of TMCFs. It has been hypothesized that water-logged TMCF soils can inhibit nutrient cycling, but also that the relatively shallow soils observed in many TMCFs can cause periodic droughts throughout the dry season (Bruijnzeel and Proctor 1995, Bruijnzeel and Veneklaas 1998). The results of Chapter 4 and 5 showed that both suggested controls are unlikely to play a role at the focal study site at 2990 m a.s.l.. Instead, the poor relationship of stomatal conductance (g_s) with VPD, SWC, or leaf water potential (Ψ_{leaf}) confirmed earlier studies in which atmospheric demand or leaf water status poorly correlated with g_s (Kapos and Tanner 1985, Cavelier 1990, Motzer *et al.* 2005). This is not to say that anticipated climate change in the tropical Andes might not change this lack of hydraulic control in C-uptake at the studied site. If the TMCFs of the Kosñipata valley experience less rainfall, or a higher atmospheric demand in the future as a consequence of higher temperatures, productivity might be affected as well through reductions in g_s. In fact, Foster (2001) pointed out that drought stress could exacerbate other effects of environmental change. Therefore, although under current conditions hydraulic controls are not important determining GPP in the studied TMCF, this can change in the future. In order to provide more insight regarding the effects of anticipated drier conditions, more (experimental) research on the drought sensitivity of TMCFs are needed.

6.6 Conclusions

The presented research in this thesis discussed different factors that can control the C-uptake of TMCFs. The main conclusions from this research are that in the

Kosñipata valley in Peru, N:P ratios suggest a limitation of P, rather than N, except at the main site that was investigated at 2990 m a.s.l.. It also showed that on a leaf level, TMCF forests have similar C-uptake capacity compared with tropical lowland rainforests when expressed on an area basis and standardised for the same temperature. However, since the mean temperatures in TMCFs are much lower than in tropical lowland forests, the *in situ* V_{cmax} and J_{max} will be lower as well, given the temperature sensitivity of the photosynthetic apparatus. The importance of temperature in controlling TMCF C-uptake was also shown by modelling gross annual C-uptake with a Plant-Soil-Atmosphere model. In this modelling exercise, TMCF GPP was about half the value found in neotropical lowland forests. Next to temperature, the incident radiation and LAI were the other two most important factors in explaining the difference between TMCF and tropical lowland GPP. Finally, both measurements in the field, as well as the modelled results indicated that under the current climatic conditions drought stress is unlikely to control productivity in the TMCF site at 2990 m a.s.l, though this might change under anticipated climate change.

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Appendix I

Supplementary information with Chapter 2

Table 1. Leaf traits of the species sampled along an altitude transect (200 – 3600 m a.s.l.) in the Kosñipata Valley, Peru. For each species four to five trees were sampled and three to four sunlit leaves form each tree were analysed. Analysed leaf traits include: leaf mass per area (*LMA*), leaf density (L_d), nitrogen and phosphorus on a mass (N_m and P_m, respectively) and area basis (N_a and P_a, respectively) and nitrogen to phosphorus ratio (N:P).

Species	Altitude	*LMA* (g m^{-2})	L_d (g cm^{-3})	N_m (%)	P_m (%)	N_a (g m^{-2})	P_a (g m^{-2})	N:P
Polylepis pauta Hieron.	3600	147	0.54	1.98	0.12	2.90	0.18	16.4
Gynoxis Rchb. sp.	3600	170	0.30	1.68	0.13	2.83	0.21	13.8
Buddleja sp.	3600	220	0.47	1.38	0.09	3.06	0.19	18.0
Pluchea sp.	3600	172	0.35	1.76	0.16	3.03	0.27	11.6
Clusia cretosa Hammel	2990	127	0.30	1.23	0.11	1.64	0.14	11.4
Weinmannia crassifolia Ruiz & Pav.	2990	134	0.31	1.31	0.11	1.76	0.15	11.7
*Schefflera allocotantha (*Harms) Frodin	2990	138	0.40	1.40	0.12	1.92	0.16	12.5
Clethra cuneata Rusby	2990	226	0.48	0.86	0.06	2.03	0.15	14.2
Clusia sp.	2350	131	0.23	1.53	0.09	2.02	0.12	16.9
Prunus sp.	2350	105	0.40	2.09	0.11	2.20	0.11	20.3
Hedyosmum scabrum Solms	2350	90.8	0.23	1.97	0.09	1.78	0.08	21.7
Miconia sp.	2350	115	0.29	2.43	0.11	2.76	0.13	22.0
Cyathea lechleri Mett.	1855	123	0.36	1.87	0.08	2.43	0.10	13.4
Clethra revoluta Ruiz & Pav.	1855	159	0.44	1.21	0.06	1.68	0.09	19.5
Myrcia sp.	1855	103	0.49	1.53	0.07	1.54	0.07	23.1
Hedyosmum racemosum Cordem. ex Baill.	1855	97.1	0.24	1.82	0.10	1.78	0.10	18.3
Mollinedia simulans J.F.Macbr.	1500	83.7	0.36	2.40	0.13	2.17	0.11	20.2
Guatteria sp.	1500	124	0.45	2.73	0.11	3.38	0.14	25.9
Tachigali cf setifera (Ducke) Zarucchi & Herend	1500	75.2	0.33	2.73	0.13	2.20	0.11	20.8
Miconia sp.	1500	94.1	0.35	2.45	0.14	2.28	0.14	18.0
Ficus sanguinosa Dugand	1000	131	0.23	1.82	0.13	2.41	0.17	14.6
Virola cf elongate Warb.	1000	66.9	0.29	2.21	0.12	1.48	0.08	18.8
Pourouma minor Benoist	1000	80.4	0.33	2.22	0.10	1.78	0.08	21.2
Pouteria sp.	1000	82.7	0.30	1.97	0.11	1.83	0.09	18.5
Leonia glycocarpa Ruiz & Pav.	220	63.9	0.33	2.14	0.15	1.37	0.09	15.2
Mabea nitida Spruce ex Benth.	220	63.1	0.43	2.71	0.15	1.84	0.10	18.5
Pourouma cecropiifolia Mart.	220	62.5	0.40	2.33	0.12	1.43	0.08	19.6
Rinorea viridifolia Rusby	220	48.2	0.37	2.34	0.13	1.12	0.06	18.8
Symphonia globulifera L.f.	220	66.9	0.42	2.29	0.12	1.56	0.08	20.6
Iryanthera juruensis Warb.	220	77.2	0.43	2.29	0.08	1.73	0.06	30.0
Bixa arborea Huber	220	62.6	0.44	2.60	0.10	1.60	0.06	26.2
Brosimum guianense Huber ex Ducke	220	66.5	0.44	2.80	0.11	1.86	0.07	25.1
Micropholis guyanensis A.D.C.	220	130	0.53	1.89	0.06	2.47	0.08	32.1
Neea divaricata Poepp. & Endl.	220	53.0	0.37	1.96	0.12	1.05	0.06	16.9

Appendix II

A-Ci curve fitting routine for use with "R". Adapted from Domingues *et al.* (2010)

```
## This script is a method for fitting a photosynthesis model to response curves of the dependency of assimilation rates over CO2 concentrations at the mesophyll.
## The method considers discontinuous nonlinear functions and solves for parameters by minimization of least-squares differences between the data and the model.
## The output parameters (Vcmax, Jmax (assuming saturating light), TPU, and Rd) are computed for temperatures measured during the response curve.
## The error term takes into account the number of observations
## Photo   = Net photosynthetic carbon assimilation   (µmol CO2 m-2 s-1)        Dependent Variable
## Ci      = [CO2] inside the leaf                    (µmol mol-1) (same as ppm or µbar)  Independent Variable
## Vcmax   = maximum carboxylation capacity           (µmol CO2 m-2 s-1)        Parameter
## Jmax    = electron transport rate                  (µmol CO2 m-2 s-1)        Parameter
## TPU     = triose phosphate utilization             (µmol CO2 m-2 s-1)        Parameter
## Rd      = respiration under light                  (µmol CO2 m-2 s-1)        Parameter
## TLeaf   = leaf temperature                         (ºC)                      Independent Variable
## Start from a "clean" R environment
        rm(list=ls(all=TRUE))
        detach()
## Load relevant libraries
        library(stats)
## Import data from a text file, skipping 12 initial information lines from a Licor 6400 standard output file. This value might change according to individual machine's setup.
        response.curve <- read.table(filename <- choose.files(), skip = 12, header = TRUE)
        attach(response.curve)
# the following prints the names of the variables from the dataset, useful to check if file was loaded correctly
        names(response.curve)
## Photosynthesis model based on:
## Farquhar G.D., von Caemmerer S. & Berry J.A.(1980). A biochemical model of photosynthetic CO2 assimilation in leaves of C3 species. Planta 149, 78-90
## Sharkey T.D., Bernacchi C.J. Farquhar G.D. & Singsaas E.L. (2007). Fitting photosynthetic carbon dioxide response curves for C3 leaves. Plant, Cell & Environment 30, 1035-1040
# Model Constants
        O2          <- 15       # Estimated Oxygen concentration at mesophyll - (kPa)
        Kc          <- 40.49    # Michaelis-Menten constant for CO2 (Pa) - von Caemmerer et al. (1994)
        delta_Kc    <- 59356    # activation energy (kJ mol-1) - Badger & Collatz (1977)
# Kc adjusted to actual leaf temperature - (Pa)
        Kc_Tleaf    <- Kc * exp((Tleaf+273.15-298.15)*delta_Kc/(298.15*8.314*(Tleaf+273.15)))
        Ko          <- 24.8     # Michaelis-Menten constant for CO2 (kPa)- von Caemmerer et al. (1994)
        delta_Ko    <- 35948                    activation energy (kJ mol-1) - Badger & Collatz (1977)
# Ko adjusted to actual leaf temperature - (kPa)
        Ko_Tleaf    <- Ko * exp((Tleaf+273.15-298.15)*delta_Ko/(298.15*8.314*(Tleaf+273.15)))
        gstar       <- 3.69     # CO2 compensation point (Pa)- von Caemmerer et al. (1994)
        delta_gstar <- 29000    # activation energy (kJ mol-1) - Jordan & Ogren (1984)
# gstar adjusted to actual leaf temperature - (Pa)
        gstar_Tleaf <- gstar * exp((Tleaf+273.15-298.15)*delta_gstar/(298.15*8.314*(Tleaf+273.15)))
        Km          <- Kc_Tleaf*(1+O2/Ko_Tleaf)
# converts Ci from ppm to Pa
        C <- Ci * Press * 0.001
## Function that contains the model equations and the variables
        ffitt <- function(x){
                Vcmax   <- x[1]     # variable 1
                Jmax    <- x[2]     # variable 2
                TPU     <- x[3]     # variable 3
                Rd      <- x[4]     # variable 4
# electron transport rate fitted to high C values
        aj <- ifelse (C >= 45, Jmax * (C - gstar_Tleaf) / (4*C + 8 * gstar_Tleaf)-Rd, 999)
# ignore intermediary C values (region of co-limitation)
        aa <- ifelse ((C > 30 & C < 45), Photo,999)
# carboxylation rate fitted to low C values
        av <- ifelse (C <= 30, Vcmax * (C - gstar_Tleaf) / (Km + C)- Rd, 999)
# triose phosphate utilization fitted to the last point of the curve
        atpu <- ifelse (C == max(C),3 * TPU - Rd, Photo)
```

```
        a <- pmin(av,aj,aa)
        diff <- sum((Photo - a)^2)                              # least-square minimization
        bdiff <- sum((Photo - atpu)^2)
        diffsum <- diff + bdiff                                 # object to be minimized
        }
## curve fitting procedure, using the "optim" command. It will vary the variables to get the smallest "diffsum" possible
## initial values (first guess) for the starting point of "optim"
        start.values1 <- c(100,130,10,3)
        f <- optim(start.values1,ffitt,control = list (maxit =9000), method = "BFGS")
## calculating the error for the curve fitting
        fit.error<-first$value[1]/(length(Photo)-sum(C>30 & C<45))
## creates a sequence of CO2 values for the figures
        Cseq <- seq(0,200,0.5)
# creates values of av based on estimated parameters (Vcmax and Rd)
        av1 <- first$par[1] * (Cseq - mean(gstar_Tleaf))/(mean(Km) + Cseq ) - first$par[4]
# creates values of af based on estimated parameters (Jmax and Rd)
        aj1 <- first$par[2] * (Cseq - mean(gstar_Tleaf))/(4*Cseq + 8*mean(gstar_Tleaf)) - first$par[4]
# creates values of atpu based on estimated parameters (TPU and Rd)
        atpu1 <- first$par[3] * 3 - first$par[4] + (Cseq /10^10)
## creating a figure showing the relationship between Photo and [CO2] at the mesophyll
        plot (C,Photo,col ="blue",pch=19,xlim=c(0,200), ylim=c(-5,60), ylab="A (µmol CO2 m-2 s-1)", xlab="[CO2] at
mesophyll-Ci (Pa)")
        title(main = c(filename),cex.main=0.8)
        lines (Cseq, av1, col = "red",       lwd = "2")
        lines (Cseq, aj1, col = "orange", lwd = "2")
        lines (Cseq, atpu1, col = "purple", lwd = "2")
        text (50,60,"Vcmax =",pos=2,col = "red");text (45,60,round(first$par[1],digits=1),pos=4,col = "red")
        text (50,55,"Jmax =",pos=2,col = "orange");text (45,55,round(first$par[2],digits=1),pos=4,col = "orange")
        text (50,50,"TPU =",pos=2,col = "purple");text (45,50,round(first$par[3],digits=1),pos=4,col = "purple")
        text (160,0,"Rd =",pos=2);text (170,0,round(first$par[4],digits=2),pos=4)
        text (160,-5,"error =",pos=2);text (170,-5,round(fit.error,digits=2),pos=4)
## end
```